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THE WISTAR INSTITUTE DEPARTMENT OF MOTION PICTURES

Film Review

A TECHNIC FOR COUNTING MOTILE SPERMATOZOA

(1 reel, 400 feet, monochrome, silent, 16 mm)

Producer: Edmond J. Farris *Distributor:* The Wistar Institute

In order to estimate male (human) fertility counts have been made, in the past, of the total number of spermatozoa, and crude estimates of the percentage of living spermatozoa in the ejaculate. Dr. Farris has developed an improved technic for making such an analysis, and has presented certain points of it in the form of a motion picture. With the aid of the "phase" microscope, which shows clearly the tails of the spermatozoa (rarely seen with the ordinary microscope) there are presented:

1. Views of normal and abnormal spermatozoa: "2-tail," "pin-head," "amorphous," and "cytoplasmic appendage."
2. Types of measurement of spermatozoa: "progressive," "sluggish," "oscillating," "circling."
3. The technic of making counts of the total number of spermatozoa and of the percentage of actively motile ones, and of measuring their rates of progression.
4. The method of obtaining, from the various data, an index of fertility. (From the figures for volume, count per cm^3, and percentage of motility is calculated the actual number of moving spermatozoa in the ejaculate.

This film will be of value to the anatomist for showing the variations in morphology and behavior of human spermatozoa; and to the clinician in determining male fertility.

Photography and legends are excellent.

Reviewed March 19, 1947, by E. R. Clark, American Association of Anatomists.

IMPLANTATION OF THE RAT EGG

II. ALTERATION IN OSMIOPHILIC EPITHELIAL LIPIDS OF THE RAT UTERUS UNDER NORMAL AND EXPERIMENTAL CONDITIONS [1]

ROLAND H. ALDEN
Division of Anatomy, University of Tennessee, Memphis

TWENTY-THREE FIGURES

INTRODUCTION

While a full understanding of events occurring during the implantation period of the mammalian embryo seems, in spite of an impressive body of literature, still to be a long way off, the problem of the interaction of maternal and embryonic elements is being investigated with increasing vigor and interest and with growing success. Whereas the aims and interests of the various studies differ to a marked degree, an underlying common denominator is discernible and is invariably associated with the transitional period when the segmenting ovum has progressed maximally as a free entity and now requires intimate association with the maternal organism for further development. That the problems and the pertinent physiological processes of this period are identical in all species would be an unwarranted assumption; yet, it is equally unlikely that differences are fundamental in character or of great magnitude. In any event, investigation of all species possible is surely desirable.

It is the purpose of this paper to report the results of an investigation of the changes in osmiophilic epithelial fats in the rat uterus during the estrous and pregnancy cycles and under certain experimental conditions. For previous reports

[1] This study was aided, in part, by a Grant-in-Aid from Sigma Xi.

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on uterine epithelial fats the reader is referred to Goldman ('12), Besse and Christides ('15), Aschheim ('15), Corner ('21), Bourg ('29), Reese ('31), Nicol ('35), Krehbiel ('37), Bloch ('39), Gillman ('40, '41), Black, et al. ('41), Aykroyd and Gatenby ('41), Rossman ('41), Gilbert ('42) and Dawson and Kosters ('44).

MATERIALS AND METHODS

Fifty female white rats raised on a standard diet were used in this investigation. Two were sacrificed when sexually immature. In 10 animals a double ovariectomy was performed at 30 days of age. The remaining 38 animals were sexually mature and were sacrificed either during certain stages of the estrous cycle or during early pregnancy (between days 1 and 7). The general procedure was to fix all or a selected piece of 1 uterine cornu in strong Flemming's fluid; the other horn was fixed in 10% formalin. When it was feasible and desirable a piece of tissue was also fixed in formol-alcohol and another in Bouin's fluid. Levi's fixative was tried, but proved less satisfactory than Flemming's for the problem at hand.

The osmicated material was stained with fast green and safranin. Formalin-fixed tissues were divided and 1 part was frozen, stained with scharlach R and mounted in glycerin; the other part was imbedded in paraffin and stained with hematoxylin-eosin. Thus in most instances it was possible to compare osmicated sections with frozen sections stained with a fat-soluble dye, and these together were compared with the usual formalin-fixed hematoxylin-stained material. Since Bloch ('39) in her study on the mouse used the classical Flemming fixative it seemed advisable, if proper comparisons were to be made, to follow her technic as closely as possible. Reese ('31), however, in his study on the rat, used a modified osmic acid technic, and some slight differences between the results reported by him for the normal estrous cycle and those given below may be attributable to differences in the program followed in the preparation of the tissues.

OBSERVATIONS

Alteration in epithelial lipids during the estrous cycle

During stage 1 (Long and Evans, '22) the epithelial cells are moderately tall, measuring between 28 and 30 microns.[2] In most cases osmiophilic granules, or droplets, are absent (Reese, '31), as shown in figure 1. However animals with a typical proestrous vaginal smear and possessing a uterus characteristically distended with fluid not uncommonly have an epithelium possessing lipid droplets of varying size and distribution (figure 2). As estrus approaches, the epithelial cells increase in height (36–38 microns) and fat appears in small amounts at the base of the cell. Only a few cells, usually in groups, possess fat (fig. 3). When the animal first comes into heat, the epithelial fat, as previously described by Reese (op. cit.), is clumped at the base of the cell, below the nucleus and near the basement membrane (fig. 4). During late estrus and early diestrus blackened droplets appear in increasing numbers above the nucleus. Very quickly they become rather evenly distributed throughout the cell (fig. 5). As diestrus approaches, the epithelium is reduced in height (25–28 microns) and the lipid material is especially abundant (fig. 6). There is a progressive decrease in the amount of fat as the next estrus approaches. In agreement with the findings of Reese (op. cit.), these preparations show that the fat is largely confined to the surface cells and only rarely does osmication or treatment with fat-soluble dyes indicate its presence in the cells of the uterine glands. Even those cells in the immediate vicinity of the gland mouth show little fat (fig. 6). Although the character and distribution of uterine epithelial fats is clearly related to the estrous cycle, the osmiophilic material appears in considerable quantity before sexual maturity. Bourg ('29) reported abundant fat in the 6-day-old mouse and figures 7 and 8 of this report illustrate the presence of epithelial lipid in rats aged 52 days and 34 days respectively.

[2] All measurements were made on osmicated material in a zone approximately mid-way between mesometrial and anti-mesometrial borders.

Alteration in epithelial lipids during early pregnancy

Although Bloch's ('39) report deals specifically with the implantation period in the mouse, only 1 figure — a drawing — showing the embryo in situ is given. This is an embryo approximately 3½ days old, situated in the egg chamber and in contact with the uterine epithelium.

In the rat up to approximately 12 hours after copulation the epithelium does not differ strikingly from that seen at estrus; the epithelium may be slightly lower (32–34 microns) and the fatty particles show a tendency to spread to the distal portion of the cell (cf. figs. 4 and 13). Intracellular vacuoles seen during stage 2 persist at least up to 18 hours after mating. At 70–80 hours, with the segmenting ova now in the uterine end of the oviduct, the epithelium is 28–30 microns tall and fat has greatly increased in amount (fig. 14). It is especially abundant basally, where the material appears to clump, but supra-nuclear particles are uniformly present. The principal difference between the appearance of the epithelium at this stage and that seen in diestrus is the greater amount of apical fat in the latter instance, with perhaps somewhat less, basally (cf. figs. 6 and 14).

At 4½ days the blastocysts are distributed along the uterine horn and may or may not be properly oriented along the implantation axis; the embryo shown in figures 15 and 18 is in the implantation chamber, but the inner cell mass is directed toward the anti-mesometrial border. This, of course, might well be due to the handling the uterus was subjected to at the time of fixation, though other embryos in this same horn are correctly oriented. The epithelium is low (20 microns) and the osmiophilic substance is distributed fairly evenly around the circumference of the lumen, as well as along the axis of the horn. Its distribution within the cell is well shown in figure 18 and the appearance of the epithelium in the interimplantation zone is shown in figure 19. While the stromal cells clearly show a reaction to the presence of the embryo, the epithelium is essentially unaltered (cf. figs. 18 and 19). Em-

bryos at this stage of development may or may not exhibit fat particles in the trophoblast layer or in cells of the inner-cell mass.

At 5½ days the epithelium in the inter-implantation zone remains essentially unchanged (fig. 21), but that in the region of the pre-implantation blastocyst is characteristically altered (figs. 16 and 20); the epithelium is low (12–14 microns) and individual cells are made out with difficulty (at the anti-mesometrial end of the implantation crypt the epithelium appears almost syncytial in character). Fat droplets appear rather uniformly coarse and become massed about the nucleus (fig. 20). In the immediate neighborhood of the blastocyst the particles show a slightly altered distribution, being scarce or wanting in the apical region of the cell. There is some evidence that the embryo shown in figures 16 and 20 has been displaced slightly (mesometrially) during fixation and that the polar trophoblast cells actually were in contact with the epithelium at one side of the implantation chamber. Although the evidence is rather more convincing in the original slide, certain features may be seen in the photographs (figs. 16 and 20): a broken stream of cytoplasm from the trophoblast to the maternal epithelium on one side of the crypt, where the epithelium is broken over a relatively fat-free area and a marked disarrangement of nuclei. It will be noted that the embryonic trophoblast shows many small, and 2 or 3 large, lipid droplets. The decidual reaction is now very evident and the stromal cells also show small amounts of fat (Krehbiel, '37).

During the sixth day the implantation process proceeds rapidly. The uterine epithelium of the inter-implantation zone is little changed; epithelial lipids may be more evenly distributed within the cell, which itself is slightly lower. In the region of the embryo marked changes are evident (figs. 17 and 22). The trophoblast tissue especially has increased in amount. The polar trophoblast cells (those opposite the inner cell mass, and nearest the antimesometrial border), identifiable on the fifth day and occasionally earlier, are greatly increased in size and number and the mesometrial trophoblast

has developed into the cap-like ectoplacental cone (Träger). Between the shoulders of the cone and the enlarged polar trophoblast the uterine epithelium has disappeared as a distinct lining of the implantation chamber and the trophoblast tissue lies against the basement membrane. (The trophoblast has, in part, peeled away from the basement membrane during fixation). Because of the osmicated fats it is not easy to distinguish trophoblast from epithelium of the anti-mesometrial crypt in these preparations; near the ectoplacental cone the terminus of the uterine epithelial layer is clearly distinguishable.

EXPERIMENTAL

There is considerable evidence that the sex hormones influence the mobilization of uterine fat (Bourg, '29; Reese, '31; Nicol, '35; Bloch, '39; Gillman, '40, '41; and Gilbert, '42). The only information available on the effect of ovarian hormones on the epithelial fat in the Muridae is the brief notation by Bourg (op. cit.) that the uterus of the castrate rat ". . . présentait un épithélium par endroits gorgé de grosses boules graisseuses . . ." and that estrone (Menformon) causes a disappearance of fat, and Bloch's (op. cit.) statement that in the mouse the ". . . secretion [fat] can be produced, when no eggs are present, by injections of synthetic corpus luteum hormone." Also, Selye and McKeown ('35) refer to basal lipoid granules in the uterine epitheluim of the rat being invariably present at the time when deciduomata may be produced and absent during periods of suspected estrin production.

Investigation of the effects of hormones on the uterine lipids has not been completed, but it seems appropriate to report at this time the results of a few preliminary experiments. Of 10 120-gram ovariectomized rats (see above), 4 were sacrificed at intervals up to 30 days; 1 was treated with estrogen alone, 2 with progesterone alone and 3 received both estrogen and progesterone.[3]

[3] Progynon and Proluton were used, through the courtesy of Dr. Gregory Stragnell, Director of the Medical Research Division, Schering Corporation.

During the first week following removal of both ovaries the uterine epithelium looks very much like that seen in the sexually immature animal. At the end of the second week there is a slight reduction in the amount of lipid, and at 30 days (fig. 9) some regions are devoid of fat, while other regions have small amounts, largely limited to the supranuclear region of the cell. It would thus appear likely that Bourg's ('29) report was based on animals sacrificed relatively soon after castration — too soon to show a reduction in the amount of osmiophilic material.

One animal, ovariectomized 2 weeks previously, was given 1500 r. u. of alpha-estradiol benzoate over 4 days. We may assume that the epithelium, at the beginning of treatment, was similar to that of an immature animal (see above); the epithelium was very probably thin (13–15 microns) with its cells filled with fat droplets. The effects of the injections were followed by vaginal smears. When the typical castrate smear was succeeded by one characterized by anucleate cornified cells the animal was killed. As figure 10 demonstrates, both epithelium and stroma responded to the hormone. The epithelium has thickened (45 microns), the cells possess rounded vesicular nuclei and no osmicated material is present. Except for this last feature, the picture compares favorably with that of a normal stage 2 (cf. figs. 10 and 3).

Progesterone administered alone ($2\frac{1}{2}$ mg divided over 5 days) produces an equally characteristic, though very different picture. Some increase in height of the epithelium is evidenced (12–14 up to 18–20 microns), but most striking is the large amount of fat that fills the cytoplasm of the cells. Basal fat is similar to that seen in early pregnancy but apical fat is abnormally increased in amount (cf. figs. 11 and 14).

Estrogen (600 r. u.) followed after 2 days by progesterone ($\frac{1}{2}$ mg daily for 5 days) produced a cornified smear which gradually gave way to one much like that seen in early pregnancy. The epithelium is tall, though not so hypertrophied as that seen when estrogen alone was given, and fat is primarily basal; however, small particles of supranuclear fat are occa-

sionally found. The picture is not unlike that seen in early pregnancy (cf. figs. 12 and 13).

DISCUSSION

Lipids appear to play a part of signal importance in the process of reproduction (Bloor, '35; Everett, '45). Although broad generalizations concerning the distribution and character of uterine lipids cannot be made unqualifiedly, there is, with a few exceptions, general agreement that fat of some kind is present in all uteri so far examined. Likewise there is considerable unanimity that it is present in greater amount during the luteal phase of the cycle and least in amount during the follicular phase. Both histochemical studies (see above) and quantitative tests (Okey et al., '30; Van Dyke and Chen, '40) are in substantial agreement on this point. The fact that uterine fat seems to disappear progressively with time in the ovariectomized animal may explain the conflicting reports of Bourg (that much fat is evident in the castrate rat), Gilbert (that little fat is present in the castrate rabbit, and this always distally located), and Nicol (that almost no fat is present in the uterus of the castrate guinea pig). It would also seem safe to conclude that fat appears normally first in the basal region of the cell and disappears last from the supranuclear region. The presence of fat in immature animals and in those in the diestrous interval goes a long way to negate the theoretical significance of the contention of Bloch that lipid (in the mouse) is ". . . present only in uteri containing blastocysts ready for implantation." One can only assume that she did not have adequate material to appreciate fully the mobilization of the osmiophilic material throughout the estrous cycle (nor did she, apparently, have access to previous reports on epithelial lipids).

Uterine fat appears in the epithelium in considerable quantity before the ovarian endocrine cycle is established; and, once this cycle functions it effects a rhythmic alteration in the amount and distribution of epithelial (and stromal) fat.

How estrogen brings about the "disappearance" of the material — as evidenced in osmicated material and that studied with fat-soluble dyes — is by no means clear (see below). While Black et al. ('41) concluded that, in the human, progesterone alone is ineffective in producing basal fat and that optimal progesterone-estrogen balance is necessary, and Nicol ('35) reports that the corpus luteum hormone is ineffective in the non-sensitized uterus of the guinea pig, in the rat progesterone alone effects a marked increase in osmiophilic epithelial lipids.

It has not been possible in this study to adduce significant support for Bloch's far-reaching conclusions concerning the role of epithelial fat in the implantation process. It is impossible to feel certain, as she apparently does, that the lipid ". . . is, without a doubt, a secretion connected with the presence of the blastocyst and proving the preparation of the endometrium for the reception of the egg." No compelling evidence was found that fat is significantly greater in quantity on the anti-mesometrial side of the uterus nor could a ". . . rhythm in the intensity of the secretion along the length of the horn" be demonstrated, either in normal animals or in castrates receiving ovarian hormones. While the osmiophilic material becomes altered at the site of implantation, it can hardly be said to "vanish," and the question of its absorption by trophoblast elements cannot yet be clearly answered; embryonic cells may very early show the presence of fat particles, which may or may not have originated in the uterine epithelium. As was mentioned previously, it seems not unlikely that the embryo shown in figures 16 and 20 actually was in early contact with the epithelium at one point, and that at the point of juncture the maternal cells are altered. It may be that some of the fat droplets seen in the trophoblast tissue involved in the union (see fig. 20) were derived from maternal cells. It is evident that 2 or 3 of the osmicated particles have the character of those seen in the epithelium and are unlike the finer droplets typical of the embryonic cells. In later stages the picture is even more suggestive of some kind of utilization

of uterine epithelial elements by the rapidly growing trophoblast (figs. 17 and 22). This important aspect of the problem of implantation is under further investigation. (Lipids, it may be recalled, can and do readily pass through the definitive placenta.)

The presence in tissues of material blackened by osmication, while presumably indicating the presence of unsaturated lipids, is widely taken as evidence of stored fats or of fatty degeneration, rarely as being indicative of secretion (Bloch, '39; cf. Rossman, '41). Diminished tissue oxidation, presumably due to interference with the blood supply, is frequently mentioned as a precursor of fatty degeneration, and the possibility must be entertained that the disappearance of fat from the epithelium of the uterus with high estrogen levels has a causal relationship with this hormone's ability to effect a heightened tissue metabolism, as suggested by the findings of Khayyal and Scott ('31), Kerly ('37), and Carroll ('42, '45). Now that the formation of fat from carbohydrate in body tissues is an accepted fact, it would be interesting to consider the relation of the glycogen and fat cycles in the endometrium. Unfortunately, an evaluation is difficult to make on the basis of current reports. It would seem that results on the human (Aykroyd and Gatenby, '41; Spyker and Fidler, '42; Hughes, '45) cannot immediately be carried over to other mammals. Krehbiel ('37) was unable to demonstrate glycogen in the epithelium of the rat uterus at any stage in the pregnancy cycle, while Wislocki et al. ('46) state that it occurs there in traces. Krehbiel (op. cit.) reported stromal glycogen developing markedly in the luteal phase of the cycle, and Boettiger ('46) finds total glycogen (as determined quantitatively by chemical methods) is nearly 4 times as abundant in proestrus as in diestrus. It may prove significant that the cycle of alkaline phosphatase is reported to be inversely related to the alterations in lipids as given here (Atkinson, personal communication). Finally, it may be that the absence and/or disappearance of osmiophilic epithelial fat does not accurately reflect the whole cycle of intracellular lipids, for

it seems not unlikely that fats may be present in another form (lipoprotein, phospholipid, etc.) in stages where they appear, upon osmication, to be totally absent. Attention is now being given to this question.

While it is clear that at this time no adequately supported conclusion can be offered regarding the functional significance of the fat content of uterine epithelial cells in the rat, it does appear that a rigorous application of histochemical and quantitative biochemical technics to this problem holds promise of throwing added light on the role of hormones in the implantation process and their possible connection with pertinent enzyme activities associated with the nidation and concomitant nutrition of the embryo.

SUMMARY

1. Epithelial lipids (those blackened by osmic acid and stained by fat soluble dyes) have been studied in the rat uterus during (a) the estrous cycle, (b) early pregnancy (to day 7), (c) immaturity, (d) in the castrate.

2. Fat increases in amount from almost none in proestrus to large amounts in the mid-interval.

3. Fat is present in considerable quantity in sexually immature animals.

4. Fat disappears progressively in the ovariectomized animal and at the end of 4 weeks osmiophilic material is reduced to scattered supranuclear droplets.

5. Estradiol benzoate causes rapid and complete disappearance of osmicatable lipids, while progesterone appears to favor storage of fat throughout the cytoplasm of the cell.

6. During early pregnancy, fat accumulates in the epithelial cells (much of it basally) throughout the length and circumference of the uterus.

7. In the interimplantation zones, the character and distribution of fat remains unaltered, but at the site of implantation its fate is obscure; there is some evidence it may be utilized by the trophoblast elements.

8. The above findings are discussed, though no conclusion is reached as to the functional significance of uterine epithelial fats.

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PLATE 1

EXPLANATION OF FIGURES

(Reduced one-third)

Magnification of all figures × 440, before reduction

1 Uterine epithelium of rat in proestrus. Note absence of osmiophilic material (fat).

2 Epithelium from rat in proestrus showing small amounts of osmicated material.

3 Epithelium from animal in early estrus. Note tall cells and scattered distribution of basal fat.

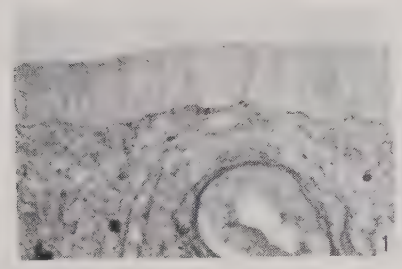
4 Epithelium in mid-estrus, showing general distribution of basal fat.

5 Epithelium during late estrus showing supra-nuclear fat.

6 Epithelium at mid-estrus showing large quantities of fat distributed throughout the cell. Note scarcity of fat in gland.

7 Epithelium from animal 52-days old.

8 Epithelium from animal 34-days old.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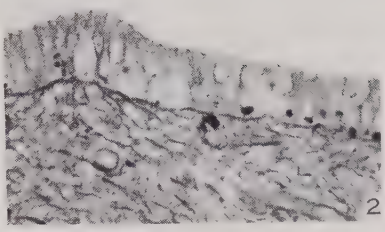
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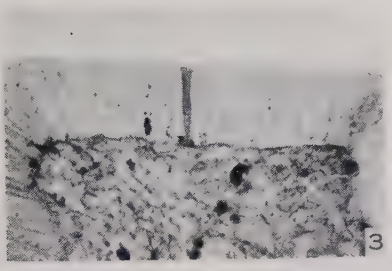
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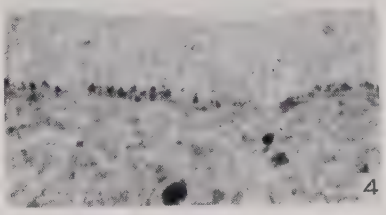
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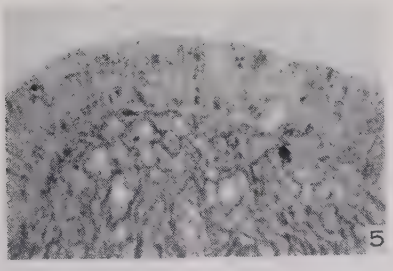
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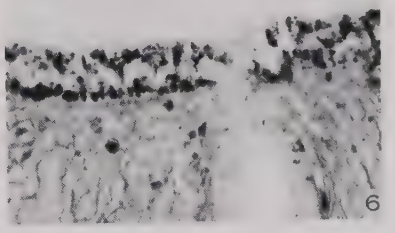
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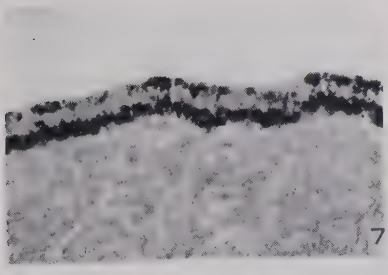
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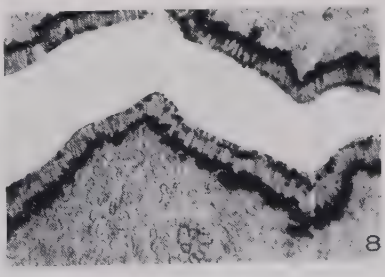
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PLATE 2

EXPLANATION OF FIGURES

(Reduced one-third)

Magnification of figures 9 through 14, × 440 before reduction
Magnification of figures 15, 16 and 17, × 150 before reduction

9 Epithelium of animal ovariectomized 30 days previously. Note irregular distribution and apical concentration of osmicated material.

10 Epithelium of animal ovariectomized 14 days previous to administration of estradiol benzoate over a 4-day period. Note hypertrophy of cells and absence of osmiophilic material.

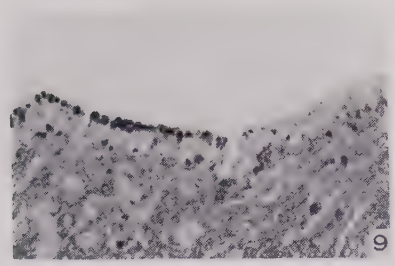
11 Epithelium of animal ovariectomized 14 days previous to administration of progesterone over a 5-day period.

12 Epithelium of animal ovariectomized 14 days previous to administration of estrogen followed by progesterone.

13 Epithelium of animal 12 hours after successful mating.

14 Epithelium of animal 70–80 hours after mating.

15, 16 and 17 Low power views of sections through the implantation sites of $4\frac{1}{2}$-, $5\frac{1}{2}$- and $6\frac{1}{2}$-day pregnancies.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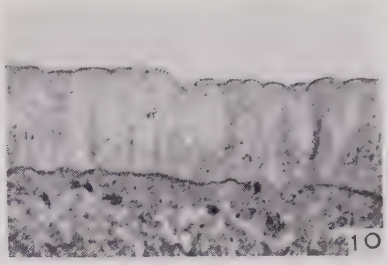
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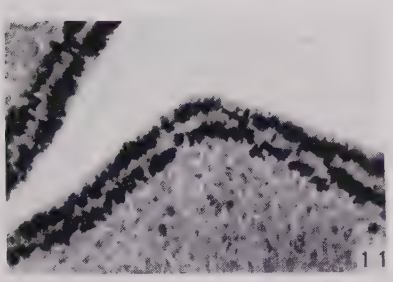
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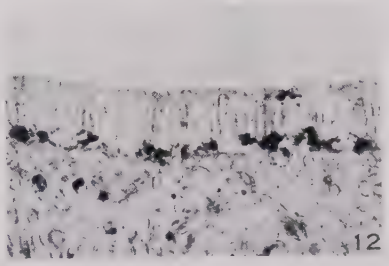
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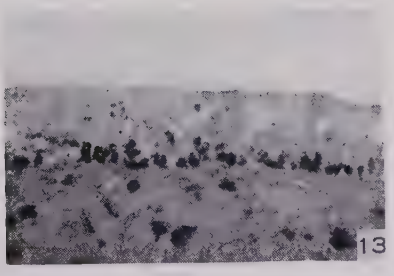
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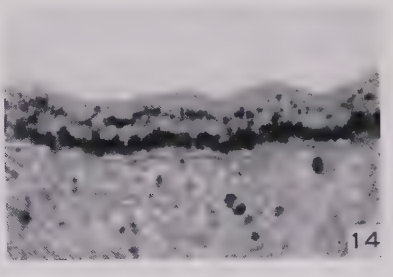
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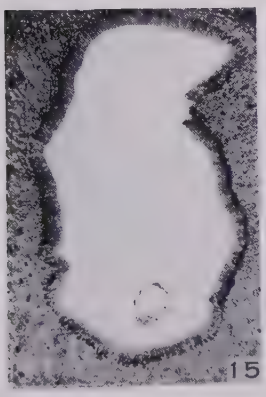
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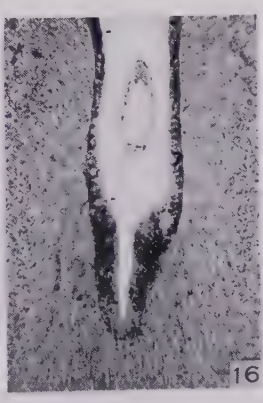
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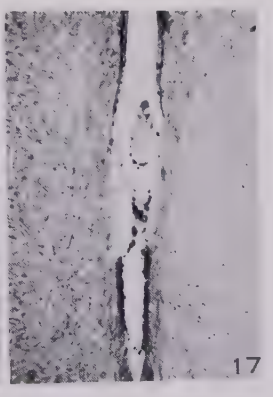
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PLATE 3

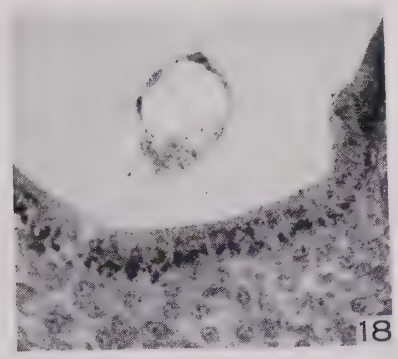
EXPLANATION OF FIGURES

(Reduced two-fifths)

Magnification of figures 18, 19, 20, 21, and 23, × 440 before reduction
Magnification of figure 22, × 150 before reduction

18, 20, and 22 Higher magnification of 4½-, 5½- and 6½-day implantation sites shown in Plate 2.

19, 21, and 23 Epithelium from inter-implantation zones of sites shown in figures opposite.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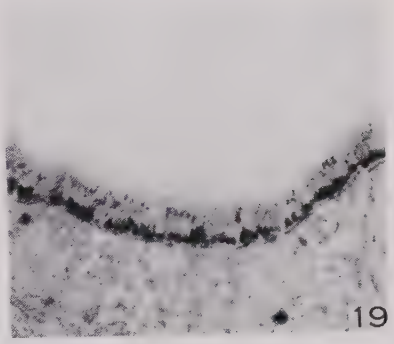
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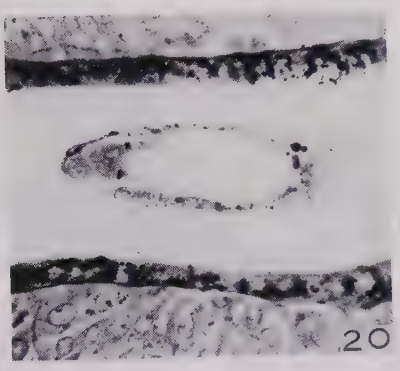
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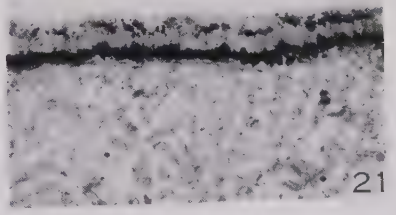
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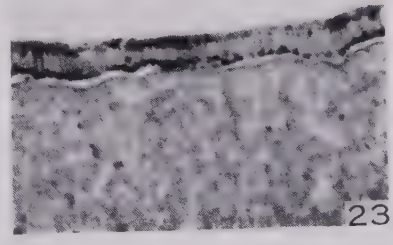
23

THE BLOCK-SURFACE METHOD OF STAINING AS APPLIED TO THE STUDY OF EMBRYOLOGY

ERLING S. HEGRE AND ALTON D. BRASHEAR
Department of Anatomy, Medical College of Virginia, Richmond

SIX FIGURES

Many students without previous training in histology and microtechnique find it difficult to interpret serially sectioned material. They are handicapped, especially in their study of embryology, by this seeming inability to establish the proper orientation of the prepared sections.

For these students the exercises to be described are recommended as an aid to them in developing a three-dimensional concept of such material. The method is based on an examination of 13-mm pig embryos which have been prepared previously as follows:

PRELIMINARY PREPARATION OF SPECIMENS[1]

The embryos are prepared by the following procedure which is a modification of a method previously described for visualizing the cut surface of specimens embedded in paraffin (Hegre and Brashear, '46a, '46b).

Specimens which have been fixed in formalin[2] and stored in alcohol are immersed in a solution of 80% alcohol in which 2.0 gm of lead acetate per 100 cm^3 has been dissolved (8 hours). They are then transferred to 95% alcohol containing 0.5 gm of lead acetate per 100 cm^3, in which solution they are left

[1] Useful variations in this procedure are given in the comments to be found at the end of this paper.

[2] Most fixatives are satisfactory. Formalin (10%) is recommended simply because it does not discolor the tissue.

over night. Dehydration is completed in absolute alcohol (8 hours, 2 changes). The specimens are cleared in a mixture of equal parts chloroform and absolute alcohol (over night) and in pure chloroform (8 hours, 2 changes). They are then infiltrated in a melted mixture of chloroform and a low melting point paraffin to which has been added about 10% white wax (over night). Infiltration is completed in a melted mixture

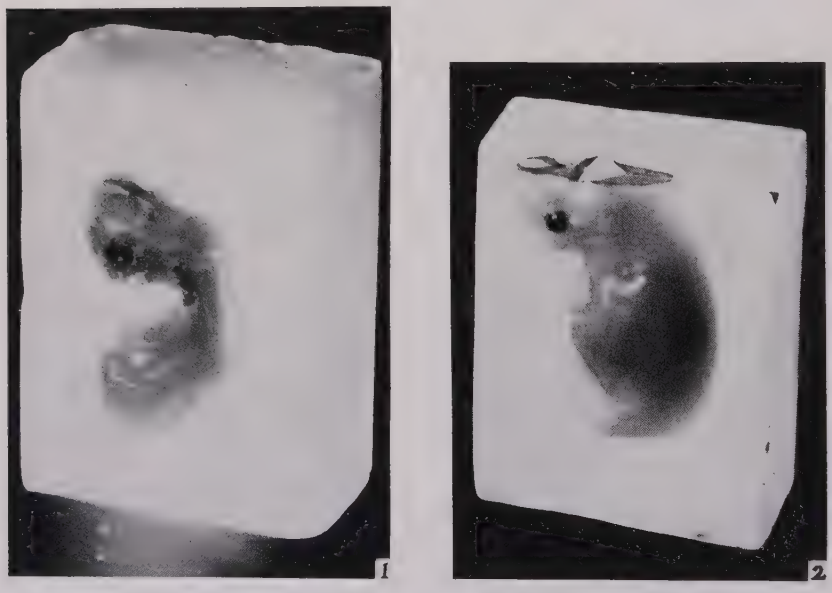

Fig. 1 Pig embryo mounted in a partially exposed position on a paraffin block. (× 2.6).

Fig. 2 Mounted specimen showing the cut surface of the block. The exposed tissue has been visualized by the application of a sodium sulphide solution. (× 2.8).

of 9 parts low melting point paraffin to 1 part white wax (6 hours with 2 changes).

The specimens are removed from the paraffin and dropped into water at room temperature. Each embryo is then mounted in a partially exposed position on a small block of waste paraffin (fig. 1).

METHOD OF STUDY

Four or 5 of these mounted embryos are prepared and issued to each student. In addition each student is provided with a simple dissecting microscope or hand lens, a safety razor blade, a few glass slides and some 5% sodium sulphide solution in a small dropping bottle with pipette.

After a review of the surface anatomy as seen on the exposed half of the embryo the student prepares to study the

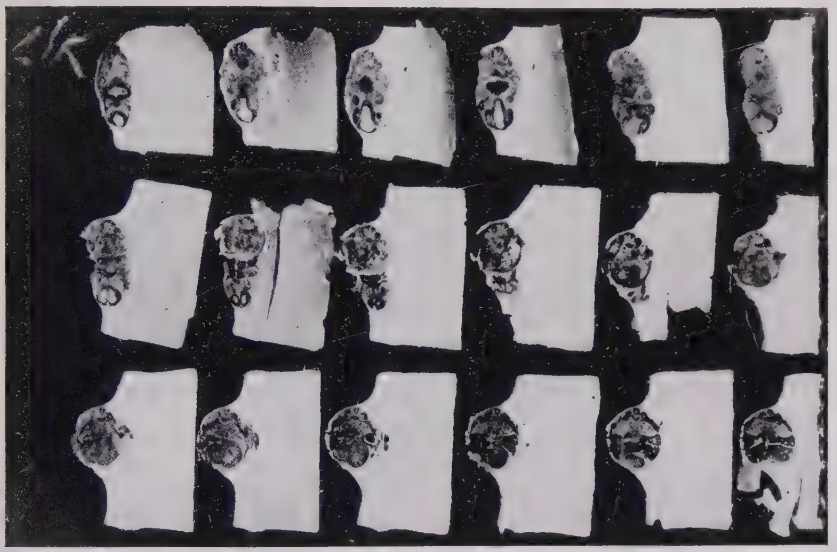

Fig. 3 Prepared slide showing part of a series of freehand sections through an embedded specimen. Each level has been stained by the block-surface method prior to sectioning. Photographed in reflected light. (× 1.4).

internal structures of the specimen in the manner to be described. First a preliminary section is made through the embryo in any prescribed plane. The tissue exposed on the cut surface is then visualized in situ by applying to it a drop of the sodium sulphide solution. The solution is permitted to act for from 5 to 15 seconds after which it is wiped off. Details on the exposed surface are then studied in their normal relation to the remainder of the specimen (fig. 2). The structures

Fig. 4 Mounted embryo showing a midsagittal exposure prepared by freehand sectioning and staining in situ, used as a reference specimen. (× 2.6).

Fig. 5 Photograph of the block surface taken directly from above. This surface was prepared on a microtone. (× 9).

are identified by consulting a standard text. Successive levels are exposed by free hand sectioning and are visualized and studied each in a similar manner.[3]

The sections prepared incidental to renewing the surface of the block are placed on a glass slide and used for further study. A serviceable preparation of the completed series can be had by warming the slide (e.g. over an ordinary substage lamp) until the sections adhere (fig. 3).[4]

[3] A tendency of the sections to curl is controlled by holding the exposed surface of the block in contact with a warm substage lamp for a moment before preparing the section.

[4] Besides being inconvenient, it is entirely unnecessary to float the sections on water (see foot note 3).

In like manner a second embryo is used for a study of sagittal levels. In the absence of a good atlas of sagittal sections the primary objective of this exercise is the preparation of a good midsagittal exposure of the specimen for purposes of reference and orientation (figs. 4 and 5).[5]

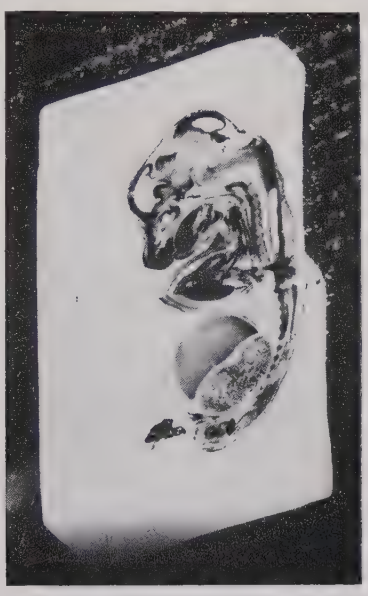

Fig. 6 Demonstration specimen showing a small fetus embedded in paraffin and exposed in three planes. The angles of the block have been retouched to bring out the three dimensional character of the specimen. (× 2).

The remaining specimens available to each student are used in a more careful study of horizontal levels or they may be used to show details in specific planes suggested by the instructor.

As special projects, the construction of demonstration specimens offers unusual possibilities since surfaces in more than one plane can be exposed (fig. 6).

[5] It would appear advisable to have the students prepare first a midsagittal exposure of a specimen to which they could later relate the horizontal levels of a second embryo. In practice this logical sequence is realized since the first embryo is often expended by the student who is quite naturally preoccupied with the technique.

COMMENTS

The time intervals given in the method for preparing the specimens were in many cases assigned arbitrarily to adapt the procedure to the average laboratory schedule. Actually a wide variation can be tolerated both as to these details as well as in the choice of some of the materials to be used. To be adequate, any modification need only provide for impregnation of the specimen with lead acetate, dehydration without removal of the impregnated lead salt, proper infiltration and embedding.

Impregnation may be carried out in a water solution of lead acetate as was originally recommended (Hegre and Brashear, '46b). However, when this is done, a 10% aqueous solution should be used since this concentration is nearly isotonic.

Dehydration can be carried out in a graduated series of alcohols provided a 10% solution of lead acetate is used in place of water in making up the dilutions. In the present method this provision was adhered to in effect by adding lead acetate to the alcohol solutions as was stated in an earlier paragraph.

Dehydration by direct transfer to dioxan is also effective. Where excessive shrinkage is likely to occur the "slow dioxan" method may be used. In this case, as was recommended for the alcohol series, a 10% solution of lead acetate should be used in place of water in making up the dilutions. It may be mentioned that with the dioxan method the tissues sometimes take on a yellowish cast. This may not be objectionable, but it is felt that for this reason and because of the difficulty in securing proper paraffin infiltration the alcohol method is to be preferred.

Chloroform has been used in the present schedule simply because it has been convenient to clear the tissue over night. Xylol is preferable in certain other respects.

In mounting the specimens care should be taken to provide a good bond of paraffin between the specimen and the block. Lack of sufficient attention to this detail is responsible for

separations that appear in certain of the sections shown in figure 3. While this does not detract from the value of the sections, it does make them more difficult to handle.

The staining time is easily controlled by direct observation and can be varied by changing the concentration of the sodium sulphide used. In general, highly concentrated and highly dilute solutions react slowly, as is to be expected. During the reaction lead sulphide is deposited in the tissue. The deposit is formed in the tissue to a depth limited by the time given for the absorption of the soluble sulphide. For the present purpose the resulting preparation is comparable to sections prepared by routine methods.

In this connection it might be mentioned that an exceedingly fine precipitate of lead sulphide is deposited when the tissue is exposed to sulphide ions in extreme dilution. This is most noticeable when the preliminary lead acetate impregnation has been reduced. Under these conditions the lead sulphide deposit is restricted to even the finest intracellular structures as seen under the high power of the microscope.

In preparing demonstration specimens embryos and small fetuses can be used. These may be cut to show as many planes or facets as is required. A part or all of a given structure may be exposed at a new depth without altering an adjacent area. Thus by a procedure not unlike dissection any desired region can be revealed. Since the stain is permanently black and is insoluble, the useful life of such a specimen is limited only by the tolerance of the paraffin.

The student exercises which have been suggested can be completed in two laboratory periods. In practice the senior author has provided his classes with facilities for making such observations at appropriate intervals during the entire course.

As has been intimated, primary significance is attached to the opportunity of examining a given prepared level in its normal relationship to the remainder of the specimen (see fig. 2). The preparation and study of serial sections in the ordi-

nary sense is therefore rather incidental.[6] This offers encouragement to the student who may find it difficult to master the technique of free hand sectioning since he cannot fail to achieve his primary objective. However, every effort should be made to preserve the series of sections intact. Thus a particular level is studied first in its normal position and secondly in its relation to previously sectioned levels. Observations made in this sequence can provide the student with a valuable first hand experience that is unique.

ACKNOWLEDGMENT

Special thanks are expressed to Dr. J. K. Finnegan of our Department of Pharmacology for his help in improving the chemical treatment of the tissue used in this method of study.

SUMMARY

An improved procedure is given for the preliminary preparation of embryos to be studied by the block-surface method.

The method of studying this material is given and has been used as the basis of a series of exercises designed to aid the beginning student of embryology in developing a three-dimensional concept of serially sectioned material.

LITERATURE CITED

HEGRE, ERLING S., AND ALTON D. BRASHEAR 1946a On visualizing the cut surface of tissue embedded in a paraffin block. Anat. Rec., *94;* 94.

———— 1946b Block-surface staining. Stain Technology, *21;* (in press).

[6] It is assumed that the students will have access to prepared histological material at least by the time they have finished these exercises.

A SIMPLE METHOD FOR MAKING PERMANENT, DIFFERENTIALLY STAINED PREPARATIONS OF SMALL NERVE PLEXUSES[1]

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While making a gross study of variation in the brachial plexus of adult turtles, the writer found that it was often difficult to distinguish between the smaller nerve connections and strands of connective tissue. A simple and quick method was sought whereby the removed plexus could be mounted and stained to allow differentiation between these tissues. It was necessary that the method be applicable to specimens which were previously preserved either in 10% formalin or 70% alcohol, for this was the nature of the material at hand.

Techniques for the demonstration of nerves previously reported in the literature, such as those of Wharton ('37) and Williams ('43), were not adaptable to the present needs. Another procedure which proved to be very satisfactory is described below. It is felt that this method might be of interest to others studying the morphology of the nerve plexuses of small animals.

The plexus is dissected from the specimen without attempting to remove all of the surrounding fascia in areas where there may be delicate nerve branches. It is then pinned out under water in a small dissecting tray, the bottom of which has been previously covered with white paper. The paper is to serve as a background for differentiation during the staining.

[1] Aided by a grant from Boston University Graduate School.

It is important at the outset to pin down all the important parts of the plexus so that the various reagents can be poured on and off without disturbing the desired relations of the nerves.

The plexus is stained with a modification of Mallory's trichrome technique. The solutions used were made according to the formulae in Mallory ('38, p. 153). The staining time found most suitable for the turtle plexus was as follows. Stain for 2 minutes in solution I (acid fuchsin). Pour off solution I, add solution II (aniline blue, orange G, phosphotungstic acid) leaving it for a few seconds, pour off and rinse the plexus quickly in water to stop the staining. The entire staining with solution II from the time it is added to the time the plexus is rinsed should not exceed 15 seconds. Admittedly, this does not permit the dyes to penetrate deeply. But these times are important, for it is essential not to get the tissues too deeply stained, and to get the nerve stained more intensely than the surrounding collagen.

If the plexus is overstained, it is best to rinse it again in order to hasten the destaining and differentiation. Continue these processes in several changes of 90% alcohol until the nerves stand out as reddish tracts fringed with blue collagen. One should aim at obtaining the correct differentiation in the smaller nerves. Care must be taken not to remove too much of the aniline blue from the tissues.

Dehydrate and harden in 95% and absolute alcohol, 10 minutes in each. If a paraffin preparation has been used as the substratum for the dissecting tray, it is desirable to transfer the plexus to a shallow dish of xylol for clearing.

Mount in clarite or balsam on a piece of window glass cut to the desired size. If the tissue has been adequately hardened, the parts of the plexus will retain their relations when it is transferred. In the present work, another piece of window glass was used as a cover, the two being held apart by small pieces of a broken microscope slide.

When such a preparation is viewed with transmitted light under a dissecting scope, it is very easy to distinguish the

smaller nerves from surrounding structures. The nerves stand out as distinct reddish-orange pathways surrounded by bluish collagen fibers. Strands of frayed fascia, which grossly might resemble nerves, are entirely blue since they lack the reddish core of nerves. The smaller blood vessels can be recognized by their contained orange-colored cells.

The chief attributes of this method are that it gives a satisfactory differentiation in material which has simply been preserved, not specifically fixed; the ingredients are readily available; and the procedure is quick and simple. After the plexus has been pinned down, the entire process can be completed in less than an hour.

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Wharton, L. R. 1937 A technique for studying the innervation of organs. Anat. Rec., *67:* 469–475.

Williams, T. W., Jr. 1943 A technique for the gross differential staining of peripheral nerves in cleared vertebrate tissue. Anat. Rec., *86:* 189–194.

A TRANSPARENT CHAMBER FOR THE OBSERVATION OF THE PANCREAS OF THE LIVING MOUSE[1]

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THREE FIGURES

The use of transparent chambers in the study of living tissue was developed by Sandison ('24), and has since been perfected by Clark and his associates ('30). Algire ('43) has recently described an adaptation of the transparent chamber technique to the mouse, and developed methods of immobilizing the mouse and the chamber.

Many observers have made microscopic studies of the living pancreas. Covell ('28), working with anesthetized mice, drew the spleen and the attached portion of the pancreas through an incision in the abdominal wall, and placed the pancreas on a warmed slide which was then covered with saline and a cover-glass. With this technique he made observations of the secretion of living acinar cells. O'Leary ('30) subsequently employed this method to study the cells of the Islets of Langerhans.

This paper describes a transparent chamber which has been utilized in the observation of the pancreas of the living unanesthetized mouse. The use of such a chamber in the study of this organ is not new, for Covell ('28) described similar chambers, made of celloidin and sewed between the layers of the muscle and skin of the anterior abdominal wall, which

[1] This investigation has been supported by the United Hospital Fund of New York City.

projected vertically for a distance of 2 or 3 cm. Portions of the pancreas were inserted in these chambers and studied by direct-transmitted light. Because of the difficulty of maintaining body temperature in such a chamber this method was not further used, and in his paper he does not describe the chamber in detail.

The apparatus described below has some of the disadvantages of that of Covell ('28), but has proved useful in the study of the blood vessels of the pancreas.

APPARATUS AND TECHNIQUE

The transparent chamber

The parts of the chamber and the details of its construction are shown in figures 1 and 2.

The chamber was made of clear "lucite"[1] obtained in sheets. The back (BC) and the base (B) were made of sheets having a thickness of 0.060 inches and the sides of a sheet measuring 0.10 inches in thickness. These sheets were shaped with small saws, files, and an electrically driven grinding tool. The sides of the chamber (S) were fastened to the base (B) with "H 94" cement[1] and dried for about 18 hours. Then the holes (H) for the screws (SC) were drilled in the walls and the holes for the sutures (HS) drilled in the base. The base was then warmed on an electrically heated hot plate until the base became flexible, and bent to the desired curved shape over a circular rod. The cover glass (CG) was cut to size with a diamond tipped glass marking pencil, and fastened in place with a glue-like mixture of cement and powdered lucite. The nuts (N) were counter-sunk in the back of the chamber and cemented in place with powdered lucite and H 94 cement. The screws (no. 80) were cut so they would not project beyond the nuts. The edges of the chamber were all carefully rounded and smoothed. The seal between the back of the walls and the

[1] "Lucite" is Methyl methacrylate resin, and both this and the "H 94" cement were obtained from the E. I. du Pont de Nemours and Co., of Arlington, New Jersey. The company also publishes a small manual giving various methods of working with "lucite."

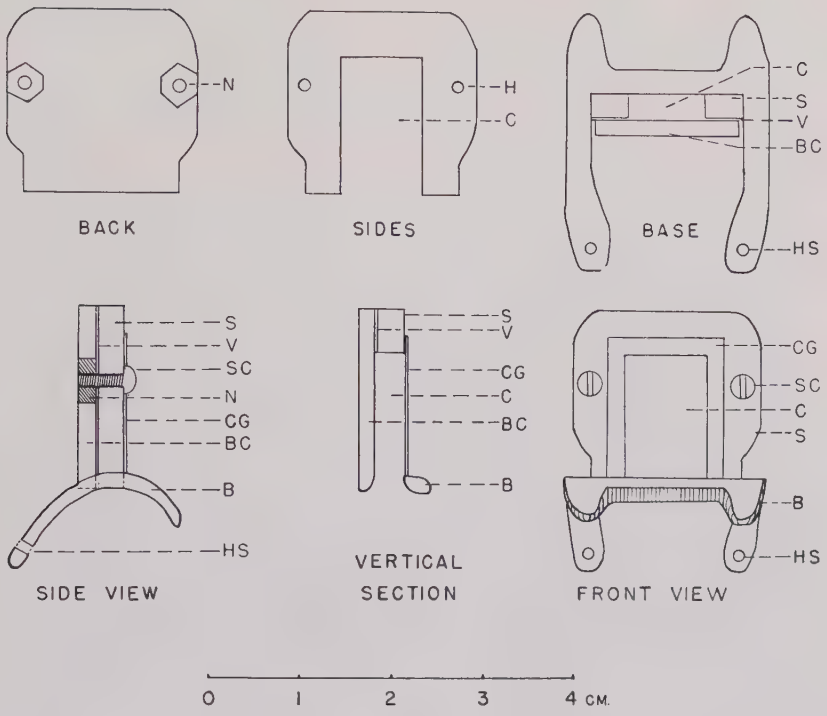

Fig. 1 Details of the Construction of the Chamber.

B, Base	HS, Hole for sutures
BC, Back of chamber	N, Nut
C, Inner chamber	S, Sides of chamber
CG, Cover glass	SC, Screw
H, Hole for screws	V, Vaseline seal

back of the chamber (BC) was made with sterile vaseline. A finished assembled chamber weighed 2.1 gm.

The support for the mouse and chamber

Algire ('43) has described a support for the mouse which immobilizes the animal effectively, and which has been used with this chamber. The support consists of a brass tube, with 1 end closed, but perforated for air, and with a slot running lengthwise which holds the chamber. The mouse is inserted

into the tube with the chamber in the slot, and the open end of the tube covered with a cap.

The support for the chamber used in these experiments was similar to that of Algire ('43), but modified to fit this chamber, and made in part of lucite.

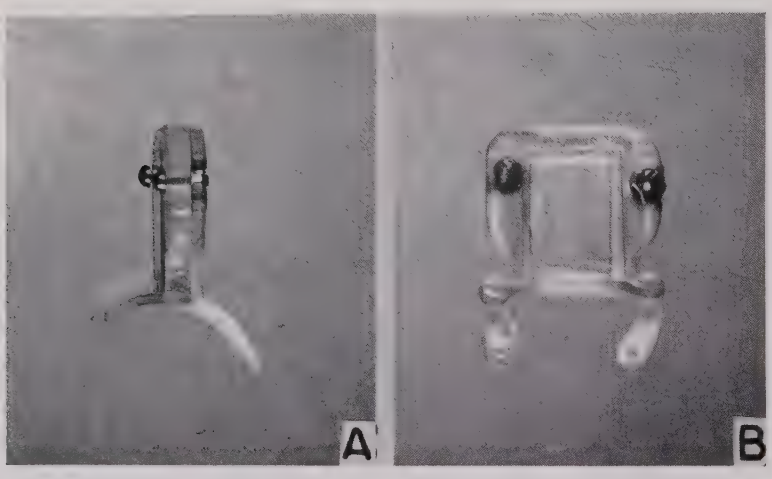

Fig. 2 Photographs of the chamber from the side (A) and from the front (B), showing the cover glass cemented in place.

The insertion of the chamber

White mice varying from 13 to 33 gm in weight have been used. The mouse was anesthetized with sodium pentothal, and its back shaved. It was then fastened with its back up on an operating board and its back was sterilized with iodine and alcohol. Aseptic precautions were observed during the instillation of the chamber, which was done in a dust-proof room.

The incision was made about 1 cm to the left of the spine and parallel to it, beginning about 5 mm above the lower margins of the ribs. The abdominal muscles and the peritoneum were incised, and the spleen and attached tail of the pancreas gently pulled out of the incision. The spleen was then dissected free from the pancreas, the large vessels which

pass from the spleen to the pancreas cauterized with a hot needle, and the spleen removed. The terminal 1½ cm or so of pancreas remained projecting from the abdominal wall. The edges of the base of the chamber (B) were then pushed between the skin and the muscles of the abdominal wall, so that the base rested on these muscles and the pancreas projected through the incision into the inner chamber (C).

Fig. 3 Living anesthetized mouse bearing the chamber.

Sutures were fastened from the holes in the base (HS) into the dense mass of tendons, fascia and muscle which lie posterior to the spine. The chamber then projected out at an angle from the back of the mouse. The pancreas was spread upon the cover slip surface of the inner chamber (C) and the back of the sides (S) ringed with sterile vaseline. The back of the chamber was then fastened into place with the screws,

and the skin edges drawn together about the chamber with sutures. In the properly inserted chamber the pancreas rests on the cover slip, and is not under pressure from the back of the chamber. A mouse carrying a chamber is shown in figure 3.

Method of observation of the pancreas

The mouse was inserted into the mouse support and placed so the chamber projected into the chamber support. A lucite rod similar in size and shape to the rod described by Algire ('43) was placed over the condenser to conduct the light to the chamber. Adequate light was obtained from a 6-volt ribbon filament lamp. A water cell and a light green filter were inserted between the light source and the microscope mirror. Observations were made with a 10 × ocular and with 25 × and 40 × water immersion objectives. As little light was used as possible. In most preparations only the thinnest portions of the tip of the pancreas were suitable for observation.

DISCUSSION

The limitations of this method of observing the pancreas are many. It is evident that the temperature of the organ is never quite the same as that in the body, though the chamber can be warmed. Degenerative changes may occur in the organ — often apparently brought on by excessive light and heat. In some preparations the circulation is slow; these should, of course, be discarded. And, finally, after about 4 days, the growth of macrophages and other connective tissue cells from the connective tissue of the pancreas into the chamber obscures the cytological details of the organ.

There are, however, advantages to this method, perhaps the most important of which is that observations can be made on the pancreas of an unanesthetized mouse. The organ itself is almost motionless, and respiratory movements do not interfere with observations. In good preparations the circulation through the organ is very brisk, and seems to be as good as that seen by the method of Covell ('28). Arterioles, venules,

and capillaries can be easily seen. It is also possible to see acinar cells, and at times Islets of Langerhans, though most of the large Islets lie deep in the body of the pancreas of the mouse and are difficult to observe.

SUMMARY

A transparent chamber has been described for the study of the pancreas of the unanesthetized mouse. Using this chamber it is possible to observe the capillaries, and to a lesser extent the parenchymal and islet cells of the living organ.

ACKNOWLEDGMENT

The author wishes to thank Miss Margaret Currie for her technical assistance.

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PERMANENT DRY PREPARATIONS OF CARTILAGE AND BONE. A METHOD ESPECIALLY APPLICABLE TO FETAL MATERIAL

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ONE FIGURE

Methods for the dry preservation of gross specimens by infiltration with various substances have been employed for many years. Specimens of brain were preserved by Hyrtl (1860) by infiltration with linseed oil and with castor oil by Fish (1893), but such preparations are greasy and collect dust readily. Permanent preparations are obtained by infiltration with nitrocellulose products (Lenhossék, 1887; Keith, '15), but considerable shrinkage of the specimens occurs. The paraffin method of Fredericq (1876) has had almost universal use and has been modified for application to many sorts of material. Rupp ('16) infiltrated organs with animal fats, but his specimens became shrunken and discolored. Recently, Kramer ('38) has succeeded in obtaining excellent preparations by the infiltration of gross specimens with a wax-like solid, diglycol stearate. The results are equal to those of Fredericq but are achieved by a simpler and more economical procedure.

Kramer recommends thorough fixation with any of the usual fluids, 4% formaldehyde being quite satisfactory. Dehydration is accomplished by 1 or 2 changes in 95% alcohol

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and is followed by infiltration with diglycol stearate [2] at 56°C. The resulting preparations retain their form and appearance for years and will withstand much handling. In the course of a recent investigation by one of us (Krahl, '46) it was necessary to prepare a number of young human bones in such a way that the cartilaginous portions would permanently retain their original form, size and appearance. The following modification of Kramer's technique yielded such excellent results in the dry preservation of cartilages that it appeared worthy of description.

METHOD

When the long bones of premature and new-born babies were fixed, dehydrated and infiltrated in accordance with Kramer's method, great shrinkage and distortion of the cartilaginous epiphyses occurred, rendering them unsuited to our needs. According to Kramer the shrinkage of gross specimens (hearts, hands, et cetera) with his method does not exceed 20%. However, shrinkage of this magnitude or even considerably less was found to markedly alter the contours and dimensions of the cartilages. Consequently, an attempt was made to determine the cause of the shrinkage and to reduce it to a minimum.

After the removal of flesh and periosteum, the bones were allowed to stand overnight or longer in a weak (1 to 2%) solution of ammonium hydroxide to remove the blood. The bones were then treated in various ways. Some were fixed for 2 or 3 days in 4% formaldehyde and then dehydrated either in 95% alcohol or were carried through a series of alcohols (35%, 70%, 95%) before infiltration. A second group received no formaldehyde fixation, but was placed directly into 95% alcohol or was taken through a series of alcohols for dehydration. All bones were finally placed in molten diglycol stearate at 56° to 57°C. for a day, more or less, depending upon size.

[2] Diglycol stearate is a cream-colored, wax-like solid, completely dispersible in hot water and soluble in hot alcohol, hydrocarbons and oils. It may be obtained from the Apollo Chemical Co., 18 Greene St., N. Y. 13, N. Y.

Bones of premature infants were well infiltrated in about 16 hours. It should be emphasized that the temperature of the molten diglycol stearate should not exceed 56° to 57°C. Higher temperatures tend to produce distortion especially in elongated pieces of cartilage (for example: costal cartilages) and marked curling takes place.

Measurements of several dimensions of the bones were taken before and after each step in the procedure. No significant shrinkage or swelling occurred during the formaldehyde fixation or while they were being dehydrated. Shrinkage in each was somewhat less than 1%. There appears to be no advantage in dehydrating with a graded series of alcohols. However, after infiltration with diglycol stearate, a marked difference was noted between the degree of shrinkage in bones fixed in formaldehyde and those dehydrated in alcohol. Those which had been placed directly into alcohol, then infiltrated often showed a shrinkage of less than 1% and never exceeded 2%. In the humeri of a 7-year-old child, no shrinkage could be measured even after they had been placed for over a week in a calcium chloride dessicator. Contrary to this, bones which had been fixed in formaldehyde exhibited great distortion of the cartilaginous portions, and shrinkage in some fetal bones was as much as 15%. It was found that freezing of the cartilages prior to treatment lead to as much as 22% shrinkage. It is evident that fixation of the cartilages with formaldehyde or freezing prior to dehydration brings about changes which end with marked shrinkage and distortion. However, when bones are dehydrated in 95% alcohol without previous fixation and infiltrated with diglycol stearate, uniformly excellent preparations are obtained.

The outline of the procedure is as follows:

1. The bones are defleshed and the periosteum is stripped off. Whiter preparations may be obtained if the bones are allowed to remain overnight in a weak (1 to 2%) solution of ammonium hydroxide.

2. Specimens are dehydrated for a suitable length of time in 95% alcohol. Cartilage of new-born individuals is suf-

ficiently dehydrated in about 24 hours. In small specimens a change of alcohol is not required since the properties of diglycol sterate are such that complete dehydration and clearing are not essential for complete infiltration.

Fig. 1 Group of bones from young humans dehydrated with alcohol and infiltrated with diglycol stearate. A. Humerus of a 7-year-old child; B. Scapula of a new-born child; C., D., and E. Humeri of 26-, 30-, and 40-week fetuses. Note the natural appearance and absence of distortion of the cartilages.

3. Infiltrate specimens in molten diglycol stearate in an oven at 56° to 57°C. The time varies with the size of the specimen. Infiltration for 24 hours is sufficient for most pieces. Bones and cartilages of premature infants are well infiltrated in 16 hours or less. The temperature should not exceed 57°C.

4. After specimens have been drained and cooled, excess diglycol stearate may be removed by rinsing them briefly in

hot water or by wiping the surface with a cloth moistened with xylol. When the excess diglycol stearate is removed the cartilages are smooth and shiny, are slippery to the touch and greatly resemble the fresh tissue. In figure 1 are seen several bones prepared in the previously described manner.

The method yields excellent results with a minimum of time and expense. It should prove of value to anatomists and anthropologists who desire to preserve fresh osteological material as dry preparations for measurement and study at a later time.

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THE CERVIX UTERI OF THE RAT

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ELEVEN FIGURES

INTRODUCTION

This study on the cervix uteri of the rat was made to determine the contributions of the cervix to the vaginal smear, or genital discharge, and to determine the histological picture of the organ under the influence of endogenous and exogenous hormones, inasmuch as no systematic work has been done on the cervix of this species.

The rat has two uteri, each of which communicates with the vagina through its own cervix and separate os cervicis. Histologically, the rat cervix is composed of two regions, a lower segment that is essentially similar to the vagina and an upper segment that is transitional between the lower cervix and the uterus. The differences between the two segments of the cervix are more apparent at certain phases of the estrous cycle than at others.

Cyclic changes in the cervix uteri have been studied in several forms, e.g. the mouse (Allen, '22); but there have been no similar studies for the rat. According to this author, during estrus the mucosa of the mouse cervix is six to eight layers thick, does not exhibit cornification, has a clear-cut basement

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Portion of thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology in the Graduate School of the University of Illinois, 1946. The portion on the cervix uteri of the rhesus monkey will appear elsewhere. (Carnegie Institution of Washington, Contributions to Embryology, 1947).

membrane, presents frequent mitoses, and contains no leucocytes. In diestrum, the epithelium of the cervix is 5 to 6 cells thick, is devoid of a basement membrane, and shows no signs of cornification. In the proestrous mouse, the cervix has a deep darkly staining and a superficial lightly staining region; the lining epithelium is 3 to 5 cells deep and is free of leucocytes.

MATERIAL AND METHOD

In the present experiments 66 rats were included. Cyclic changes in the cervix were studied in 15 estrous, 14 metestrous, 11 diestrous, and 10 proestrous rats. Four pregnant, 3 pseudopregnant and 9 estrin-treated rats were also used. Except for a few hooded and hairless rats devoted to the study of the estrous cycle, all of the rats were albinos.

In the study of the cervix during the estrous cycle, the rats were killed with ether; immediately upon death vaginal smears were taken. The rats were then opened and the cervix dissected out. The cervices were studied by the following methods: (1) Smears were made of the uterine end of the cervices by means of tufts of cotton on wooden applicators. (2) Some genital tracts were opened their full lengths, flattened out, and the mucosa pressed against a glass slide. In this way an imprint was made of the whole cervical as well as the adjacent vaginal and uterine regions. These smears were stained with Shorr's vaginal smear stain after fixing in a mixture of equal parts of 95% alcohol and ether (Shorr, '40). (3) A number of the flattened, open cervices were preserved in Carnoy for in toto staining with Shorr's stain. (4) Intact cervices were preserved either in Bouin's, Carnoy's or A.F.A. fixing solutions; for subsequent sectioning and staining Harris' haemotoxylin and eosin and Shorr's vaginal smear stain were employed. Five μ was found to be the most satisfactory thickness for the Shorr stain. Some of the rats were carefully staged by means of daily smear examinations over a number of estrous cycles; the positions in the cycle of animals that had to be sacrificed without such prior examina-

tions were estimated from a single vaginal smear taken at the time of killing, admittedly a possible source of error.

The pregnant, pseudopregnant, and estrin-treated rats were vascularly injected with India ink at the time of death. Pseudopregnancy was established by electrical stimulation of the cervix at estrus. These cervices had been cleared in oil of wintergreen for the study of the circulatory system by Miss Maude Williams and were generously made available by her for this study. It was found that soaking the unduly hardened specimens in 50% alcohol for several hours rendered them sufficiently soft for sectioning.

The estrogen-treated rats were divided into two groups; immature intact females and ovariectomized females. The immature 21-day old females each received 0.1 γ estrogen and were killed after 12, 30, and 54 hours. An untreated animal from each group served as control.

With Shorr's vaginal smear stain, the cornified elements take on a deep orange color, mucous cells pale green and occasionally light orange, epithelial cells green generally, and deep epithelial cells red. In the discussion of the vaginal and cervical smears, the cells will be described in these terms.

The rats for the study of the cervix during the estrous cycle were such a heterogenous assortment of strains that some variation in mucosal height could be expected. Rats from an inbred strain might be more uniform in such a character.

OBSERVATIONS

1. The cervix of the rat during estrus

The cervical smear at this stage did not show a characteristic cellular picture. Mucous strands, a few scales, some epithelial cells, and an occasional leucocyte were seen. The smear made by pressing the cervix against a slide showed numerous scales in the part against which the lower cervix had been impressed; above this in the differentiated region of the cervix, to be described below, only a few mucous strands were seen.

The total mount of the estrous cervix stained with Shorr stain presented a significant picture. The vaginal portion and also the vaginal fornices stained bright orange as did the lower half of the cervix, which strikingly demonstrated that this lower area of the cervix was covered with cornified epithelium. The upper cervix stained bluish-green, more or less definitely due to a green epithelial layer above and a blue deeper layer. The cervix was thrown into gentle longitudinal furrows; the mucosa had a soft velvety appearance.

Sections of the cervix varied somewhat, depending upon which part of estrus, early or late, a given rat was in. The mucosa was hyperemic and 7 cells deep. In the sections, as in the whole mounts, it was found that there was a sharp differentiation between the upper and lower cervix, the latter having cornified strata. The proprial margin in the lower cervix was very distinct and had a saw-toothed border. The stratum germinativum was composed of many small cells; mitoses evidenced growth of the organ at this stage. The keratinized layers of the cervix desquamated rather early in estrus, and the mucosa was thin in consequence. This metestrous condition appeared in the cervix before vaginal desquamation had been completed. Before denudation had commenced, columnar epithelium had been formed under the keratinized layers (fig. 1). The uterine end of the cervix was characterized by a less distinct basement membrane than was the lower, and the proprial border was smoother. There were no cornified strata and the canal lumen was lined by columnar epithelium. The wall of the upper cervix was infiltrated with vast quantities of leucocytes; these leucocytes are probably released during the metestrum (fig. 2). At the site of the internal os, there was a ring-like thickening of the cervical mucosa.

The cervical mucosa in the region of the external os ranged from 36 to 127 μ — before desquamation, 54, 68, 86, 127 μ; after desquamation, 36 μ. In the non-keratinized upper cervix the mucosa was generally between 50 and 60 μ thick. The hairless rat in this group had a much thinner mucosa, at the

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external os measuring 20 μ and at the cervico-uterine junction, 11.4 μ.

By way of summary: the rat cervix during estrus has been shown to have two well differentiated areas, a lower one with cornified scales and an upper one that has a layer of columnar epithelium innermost. The scales produced in the lower cervix probably became a part of the genital discharge and appear as an element of the vaginal smear, a fact not heretofore emphasized.

2. Cervix of the rat during metestrum

No striking feature characterized the cervical smear or the total mount at this stage. Mucous strands, mucoid epithelial cells, and a few deep epithelial cells were seen but were not present in abundance.

Sections of the rat cervix during metestrum show that there was little differentiation between the upper and lower cervix except in depth of the mucosa, as contrasted to the condition during estrus. The epithelium of the metestrous cervix was 6- or 7-cell layers high and was not edematous. There was a distinct basement membrane; and the stratum germinativum gave no indication of mitotic activity. The peripheral strata were composed of stratified squamous cells whose nuclei were large and round and surrounded by a moderate amount of cytoplasm. The epithelial layer bordering the canal was cuboidal to columnar, and the cells appeared filled with a mucoid substance. Some leucocytes were working their way through the cervical wall (fig. 3). One preparation of the cervix during early metestrum had mucified stratified epithelium with an inner layer next to the lumen of cuboidal mucoid cells. In some rats leucocytes were extremely profuse, and the mucification of the epithelium was more extensive. Deep red-staining epithelial cells were seen in the cervices of some of the rats; these may be the deep red-staining epithelial cells that are found in subsequent days in the vaginal discharge (Hartman, '44).

The cervical mucosa in the region of the external os varied from 20 to 54 μ in height. At the internal os, the heights ranged from 18 to 50 μ. In each rat, the mucosa was somewhat thicker at the external os than at the internal os, although its general appearance was the same.

In summary, metestrum was characterized by a rather inactive cervix which was emitting a few leucocytes and mucous cells. There was no sharp differentiation between the upper and lower regions of the cervix.

3. Cervix of the rat during diestrum

The paucity of cells of the preceding stage continued in the diestrous cervical smear. It contained some pale bluish-green mucous cells, leucocytes, and mucous strands, but only in small quantities.

The whole mount of the tract exhibited some cornification in the vagina, but the cervix showed superficial green staining cells which represented the mucified columnar epithelium bordering the cervical canal; underneath this haematoxylin stained strata were evident.

A study of microscopic preparations showed that the upper and lower cervical segments were essentially similar. The mucosa was around 5-cell layers thick, and in contrast to the cervix at estrus, it was compact and not hyperemic. The basement membrane was not conspicuous in any portion of the cervix. The proprial margin was moderately smooth with some small protrusions or sprouts from the stratum germinativum whose nuclei were not as small nor as abundant as in the external os region of an estrous animal and were in closer conjunction with each other as the cells were smaller. There were typically several layers of stratified squamous epithelium with a columnar layer next to the lumen, although in some cases this columnar epithelium had been reduced to a cuboidal one. Leucocytes were quite abundant in the cervical wall; mucous strands, mucous cells, and leucocytes were found in the lumina of the cervical canals of all the rats in this

group. Red or deep epithelial cells occurred in the mucosa of some of the rats (fig. 4).

The mucosa was thicker in the region of the external os than at the cervico-uterine junction. At the os cervicis the average thickness was between 25 and 65 μ with the greatest number of rats measuring 45 μ. At the internal os, the height varied from 14 to 36 μ, with the majority around 20 μ.

In summary, the diestrous cervix contributed mucus, small green cells, a few deep red-staining epithelial cells, and many leucocytes to the genital discharge. The upper and lower regions of the cervix were similar in appearance.

4. Cervix of the rat during proestrum

Cervical smears again showed only a few cells. Mucous strands and mucous cells were seen in moderate abundance, in some cases. The smear made by pressing the cervix against a slide included a number of green epithelial cells; these may represent the epithelial cells of Type VI, Class 6, as described by Hartman ('44). Pale-orange epithelial and mucous cells, a few scales, and some leucocytes also were seen in the smear.

A whole mount of the cervix stained in toto showed both the vagina and cervices to be covered with green-staining epithelial or mucous layers. In the vaginal regions and also in the lower cervical regions cornification seemed to have commenced, for an orange cast of the underlying strata was apparent through the overlying greenish layer, an interpretation strengthened by the sections next to be described.

Differentiation between the upper and lower cervical segments was becoming evident. The cervical mucosa was around 8-cell layers deep. There was no distinct basement membrane, and the stroma and mucosa were becoming hyperemic. The proprial strata were composed of several layers of small cells with indistinct cell borders and small nuclei which were not pycnotic; these small cells later assumed more superficial positions in the mucosa replacing cells sloughed off. Next

peripherally were layers of squamous cells with keratinization apparently occurring in the lower cervix in the cytoplasm of the upper 3 or 4 layers. The nuclei of the cornifying cells were degenerating, and these cells formed a darkly staining deeper stratum. The layer next the lumen was made up of mucoid columnar epithelial cells which stained pale green with the Shorr stain. The nuclei of these columnar cells were oval and rather dark. Leucocytes were abundant (fig. 5).

Rats that were adjudged to be in a late proestrous state had already lost their superficial lightly staining layer and the underlying stratum was definitely cornified in the lower cervical segment.

The upper cervix rather resembled a diestrous cervix as this did not have a region of keratinizing cells with pycnotic nuclei. The superficial lightly staining columnar epithelium was quite apparent in the upper cervix (fig. 6).

The cervical mucosa measured from 37 to 164 μ at the external os, and from 45 to 196 μ at the internal os. It was thinner in the late proestrum due to the loss of the mucified layer. It was also thinner in very early proestrum due to the fact that cellular proliferation was not yet active and edema had not been established. As a rule, the internal os region was thicker than the external os region.

By way of summary, the proestrous cervix had a superficial lightly staining region which was lost before estrus and became part of the genital discharge, and a deeper darkly staining region that cornified. The differentiation between the upper and lower cervix was beginning to be manifested.

5. Cervix of the rat during pregnancy

On the fourth day after fertile mating, the rat did not yet exhibit mucification of the vagina, which is characteristic of pregnancy. There were some scales in the lumen of the vagina. The cervix showed sloughing of an inner layer of low columnar epithelial cells; just below the desquamating region there were layers of flattened, possibly cornified cells, and

below this were several layers of rounded epithelial cells. The stratum germinativum was in a state of active proliferation.

On the seventh day of pregnancy mucification was evident. Tall columnar cells with basal nuclei lined the cervical canal; there was some mucus found in the canal lumina, and the stratum germinativum was active. In the vagina mucification had not yet progressed greatly.

Day 12 of pregnancy showed a high degree of mucification in vagina and cervix. Leucocytic infiltration was spectacular in both the lumina and the walls of the cervix and vagina. The mucified layer in the cervix was much higher than in the preceding case. The cervix had more straight glands filled with mucus than on the fourth day of pregnancy, when they were beginning to be formed.

On day 16 of pregnancy, mucification was extreme both in the cervix and vagina, although much the greater in the latter. The cervical mucosa was made up of tall mucous cells with large globules of mucus and nuclei in a variety of situations in these cells. The lower strata, as in the last 2 cases, were made up of rounded epithelial cells; the stratum germinativum consisted of rather small closely packed cells.

In summary, the cervix underwent pregnancy alterations which involved cellular proliferation of the mucosa, mucification of the innermost strata, and increase in the depth of the mucosa. The changes were similar both in the upper and lower segments of the cervix, but the extent of mucification and development of the straight glands was considerably greater in the lower cervix than in the upper.

6. Cervix of the rat during pseudopregnancy

On day 4 in the pseudopregnancy cycle, the vagina exhibited little evidence of mucification, appearing not dissimilar to the condition in proestrum. The cervix had an inner layer of columnar cells with basal nuclei. The cervical mucosa was 3- or 4-cell layers thick and as yet exhibited little alteration. The picture was the same as in the pregnancy cycle.

Day 7 of pseudopregnancy showed a more pronounced vaginal mucification. The cervical canals were lined by rather tall columnar cells with distinct mucoid globules and nuclei still mainly basal. Cellular proliferation was proceeding in the stratum germinativum. Again the picture was directly comparable to pregnancy at the same stage.

By the twelfth day of pseudopregnancy, the vagina was extremely mucified. The cervix presented a similar but less pronounced picture. Leucocytes were present. Tall columnar mucus-laden cells with variously placed nuclei lined the cervical canals, and there were straight, unbranched mucus-filled glands. The lower strata of the cervical mucosa were made of 4 layers of epithelial cells in addition to the stratum germinativum.

In summary, pseudopregnancy was characterized in the rat cervix by a gradually mounting mucification of the inner stratum of the mucosa through day 12, after which time, on the average, regression had set in. These alterations were identical to those observed in the pregnancy cycle through the twelfth day and were similar in the upper and lower cervical segments, althought more pronounced in the latter.

7. Cervix of the rat in estrogen treated females

a. In ovariectomized females. In the untreated ovariectomized female, the cervix as well as the remainder of the reproductive tract was in a state of regression. The cervical epithelium was 2 cell-layers thick, with a layer of cuboidal cells around the cervical lumina and an underlying stratum of small cells containing small oval nuclei that were placed parallel to the canal lumina. The basement membrane was not distinct (fig. 7).

Six hours after the injection of 0.001 mg estrogen, proliferation in the cervix was apparent. The stratum germinativum was composed of numerous small, oval cells lying with their long axis perpendicular to the canal lumen. The cells bordering the canal were still cuboidal in shape, having altered little.

The main new development in the mucosa consisted of the mitotic activity of the stratum germinativum and the 45-degree rotation of the nuclei. The basement membrane was still indistinct. The stroma was more cellular and more vascular (fig. 8).

By 12 hours there was a mucosa of 3- or 4-cell layers in a rather loose arrangement. Crypts into the propria had been formed, and the basement membrane was not much more distinct than at the preceding stage. The cells of the stratum germinativum were much larger than in the previous stages and were still quite numerous. The cells lining the canal had a shallow cuboidal shape (fig. 9).

In 30 hours, a picture similar to that in proestrum was seen. There was a differentiation of the mucosa into 2 regions, a superficial lightly staining and dark underlying layers. Cornification was beginning in the darkly staining region in the lower cervix. There was a distinct basement membrane and a differentiated stratum germinativum. The mucosa was about 5-cell layers thick. The superficial lightly staining region had large cuboidal cells with vesicular nuclei. This was the first stage in which the upper cervical segment was well differentiated from the lower, inasmuch as the upper segment did not exhibit a cornified strata, a distinction that it attained in the proestrum of the normal cycle (fig. 10).

After 54 hours the cervix had progressed to the estrous stage of a normal cycle. The mucosa was 6- or 7-cell layers thick and was edematous. Cornified strata with a distinct basement membrane and papillary processes, indicating proliferation, had developed. Most characteristic of all, the lower and upper portions of the cervix had reached maximal differentiation (fig. 11).

b. In immature females. The control of this group, i.e. the untreated 21-day old female, had a cervix with a mucosa of 3- or 4-cell layers and an already differentiated stratum germinativum, as in the 12-hour animal above. The layer of cells bordering the canal was composed of low cuboidal cells.

By 12 hours after the injection of 0.1 γ of estrogen, the mucosa was already thicker. The cell layers had a loose appearance and were rounded rather than squamous. The basement membrane was not distinct, the stratum germinativum was composed of numerous rather large cells, and cuboidal cells lined the canals.

After 30 hours the cervical epithelium had become 4- or 5-cell layers deep. The cells by now had assumed a squamous shape but cornification had not yet been established. The superficial lightly staining region was in the process of being sloughed. There was a very definite basement membrane. The lower cervical region was more developed than the upper.

The 54-hour specimen showed a cervix that was quite similar to that of the ovariectomized female after 54 hours, but with somewhat less cornification. Likewise, the upper and lower cervical segments were well differentiated from each other, with the upper lacking a keratinized layer and having a less distinct basement membrane.

In summary, estrogen caused an estrous reaction in the cervix of the ovariectomized adult and in the intact immature rat. Comparing the two classes of test animals it appears that the effect of estrogen went further, i.e. was more marked and lasted longer, in the castrated adult than in the immature animal.

DISCUSSION

The cervix of the rat exhibited a gradient from the vagina to the uterus both in structure and in its response to estrogen. Like the vagina, the cervix was lined by stratified squamous epithelium, but it was not as thick, being intermediate and transitional between the vaginal mucosa and the uterine endometrium.

At estrus, presumably the time of greatest estrin production, the cervix reacted by heightening of the epithelial cells and edema of the epithelium and stroma, with this effect being more pronounced in the lower half. This was similar to the effect of estrin in the monkey cervix (C. Hamilton, in press).

Estrin was also active in stimulating the cellular proliferation of the stratum germinativum. In whole mounts of the longitudinally split cervices stained with Shorr's stain, the 2 regions of the organ stood out in bold contrast, the lower being orange in color (indicative of cornification) and the upper blue-green and uncornified. A preparation made by pressing the whole mucosa of the split genital tract against a slide yielded a similar result. Some of the scales in a vaginal smear taken during estrus were very probably cervical in origin, a possibility not hertofore generally recognized.

Metestrum marked the conclusion of regression of the reproductive tract. At this period, the cervix was rather inactive and contributed only a few leucocytes and a few epithelial and mucous cells to the genital discharge. Desquamation seemed to increase towards the end of the phase with the increase in mucous cells. There was no sharp differentiation between the upper and lower regions of the cervix at this time.

Diestrum or the interval phase showed a mucified cervix which added great swarms of leucocytes and mucous cells to the vaginal discharge. The upper and lower cervical regions were similar in appearance during diestrum. Towards the end of diestrum, epithelial cells in the mucosa began staining red with Shorr's vaginal smear stain; these may be at least a part of the deep epithelial cells that appear in the vaginal discharge during proestrum.

The proestrus picture showed an increase in the bulk of the cervix and a beginning of differentiation between the lower and upper segments, due to the increasing estrin level. The contributions of the cervix to the proestrous vaginal smear probably consisted of epithelial and mucous cells and a few leucocytes. The superficial lighter staining layer was lost before estrus and became a part of the genital discharge.

The present study therefore tends to confirm Hartman's ('44) suspicion that certain cells found in the vaginal smear and differentiated by means of Shorr's stain were of cervical and not of vaginal origin. Such are almost certainly his

"Epithelial Type VI, Class 6," namely small light green cells with pycnotic nuclei, often found entangled in mucous strands along with clouds of leucocytes. Hartman's "red epithelials" of Class 3 are probably also cervical in origin.

During pregnancy and pseudopregnancy, the cervix exhibited changes similar to those of the vagina, but of a lower grade. Whether these alterations were due to the progesterone from the corpus luteum or to the low concentrations of estrin formed by it even during the early stages of pregnancy is still uncertain. The changes that occurred (cellular proliferation, increase in height of epithelium, mucus formation) are all changes that had been demonstrated in the body of this paper to be due to the action of estrin.

After treatment of either castrated or immature female rats with estrin, the cervix presented an estrous picture after 48 or 54 hours, in agreement with the results found by Allen in the rat uterus and vagina. The process was gradual and the reaction time was the same in the ovariectomized and immature animals, but in the latter the effect was less pronounced and of shorter duration. The immature control animal had a cervix that was less involuted than that of the ovariectomized adult female showing that there was already estrin production in female rats of 21 days of age.

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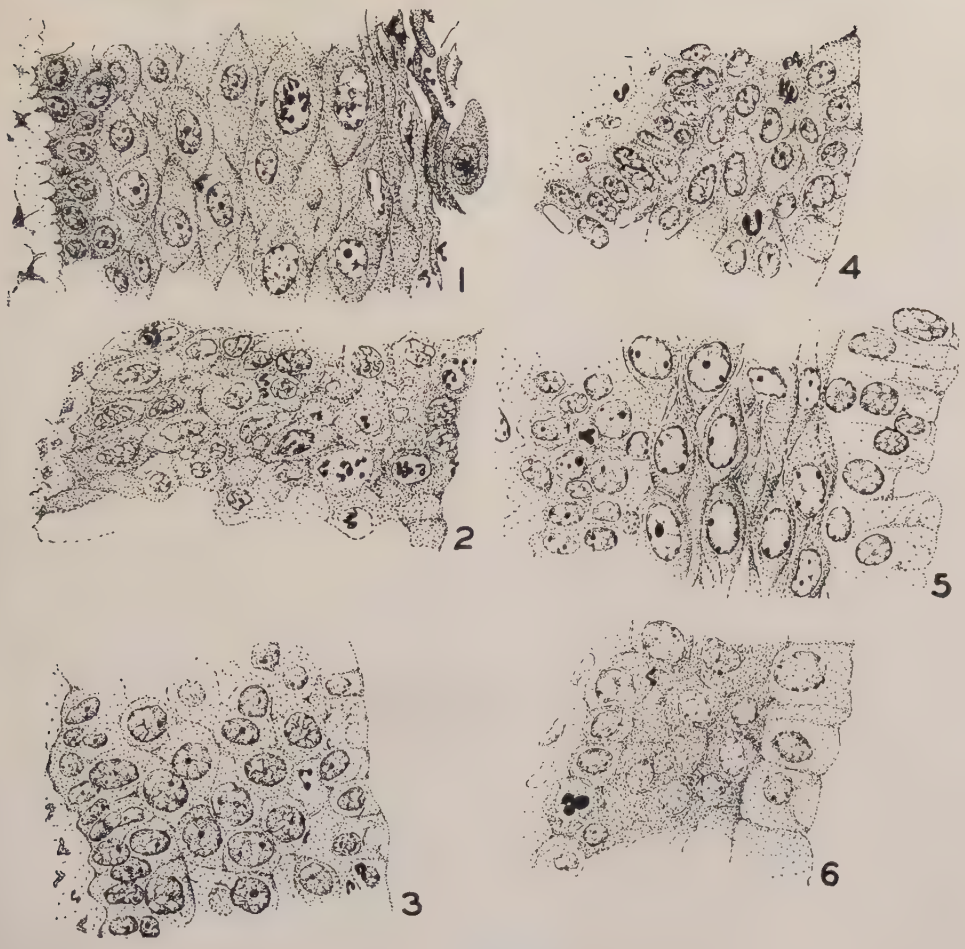


Cervix of the rat during estrous cycle (× 910).

1 Estrus. Section near os cervicis showing edematous mucosa, sloughing of cornified cells, layers of epithelial cells formed to replace the desquamating cells, active proliferation of stratum germinativum, and a distinct and uneven basement membrane.

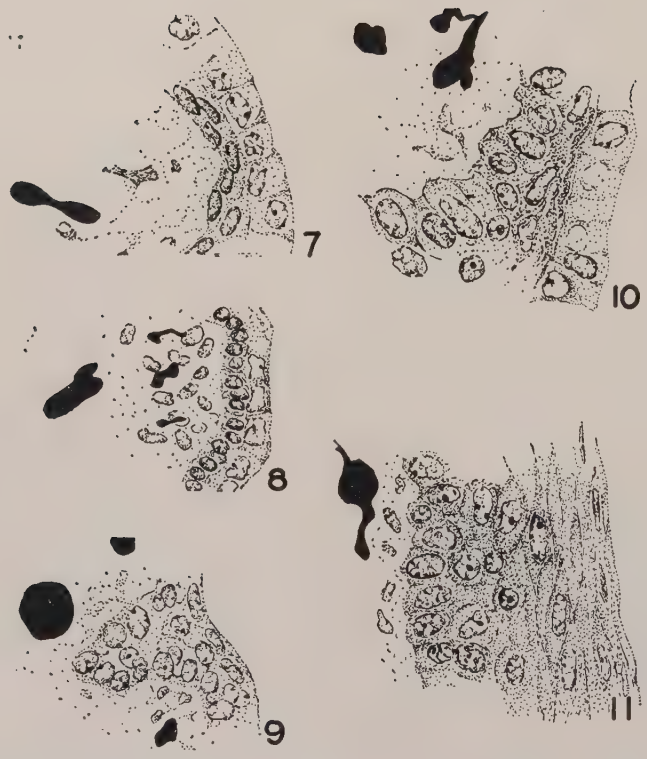
2 Estrus. Section near cervico-uterine junction showing heavy leucocyte infiltration, thick edematous mucosa, non-cornified cells, and less distinct basement membrane.

3 Metestrum. Degenerative stage showing a superficial layer of cuboidal epithelial cells over several layers of only slightly edematous epithelial cells, and a distinct basement membrane.

4 Diestrum. Interval stages showing a non-edematous mucosa with abundant leucocytes, proliferating stratum germinativum and an indistinct basement membrane.

5 Proestrum. Section near os cervicis showing tall columnar cells forming a lightly staining surface layer darkly staining cornifying cells, a region of developing epithelial cells, an indistinct basement membrane and an increasing edema.

6 Proestrum. Section near cervico-uterine junction showing similar regions as figure 5, but lacking cornified strata.



Cervix of ovariectomized rat after estrogen treatment (× 910).

7 Control. A typical involuted cervix.

8 Six hours after injection. The stratum germinativum has undergone proliferation.

9 Twelve hours after injection. The cervix is now composed of 3 or 4-cell layers and beginning epithelial downgrowths.

10 Thirty hours after injection, similar to a proestrous cervix.

11 Fifty-four hours after injection, similar to an estrous cervix.

MITOTIC DIVISION AND DEGENERATION OF LYMPHOCYTES WITHIN CELLS OF INTESTINAL EPITHELIUM IN YOUNG AND IN ADULT WHITE MICE [1]

WARREN ANDREW AND JULIO MARIA SOSA

NINETEEN FIGURES

INTRODUCTION

The cytological processes dealt with in this report form the subject of an earlier paper (Andrew and Andrew, '45). In this first study we dealt with the passage of lymphocytes through duodenal epithelium in a group of ten black mice of the Strain C57 from the Jackson Memorial Laboratory at Bar Harbor, Maine. These animals varied in age from 110 days up to and including senility, e.g., 600 days and over. In all of them very large numbers of lymphocytes were found in the epithelium both of the crypts of Lieberkühn and of the villi. A number of features of these lymphocytes and of their relation to the epithelial cells led us to conclude that the majority of them were actually intracellular in position. A series of changes in the lymphocyte during its passage from base to apex of the epithelial cell was described. Of signal interest is the fact that in the crypts many of these cells are undergoing mitosis, at times normal, at other times aberrant. It was suggested that the most probable significance of the degeneration and of the mitosis is some type of defensive

[1] The work reported here was carried out in the Institute of Neurology and in the Institute for Biological Sciences, during the period from the eighth of September of 1944 to the eighth of January of 1945, while Professor Andrew was serving as "Visiting Professor" from the Department of State of the United States and as a guest of the University of Montevideo, in order to carry out investigative studies in collaboration with Professor Sosa. Dr. Andrew is at present Professor of Histology and Embryology at Southwestern Medical College, Dallas, Texas. Dr. Sosa is carrying on investigative work in the Department of Anatomy, University of London, under a British Research Council grant.

process, and the hypothesis was put forward that this process may be similar to that seen in the "reaction centers" of Hellman ("germinal centers" of Flemming). The degenerative and phagocytic processes in such centers were described and their significance discussed in another paper by one of us (Andrew, '46).

The present study has been made in an attempt first, to confirm in another strain of mice the findings previously described by us; second, to make more detailed cytological observations on the processes by means not used in the earlier study; and, third, to ascertain the conditions obtaining in regard to lymphocyte-epithelial relation in the early life history of the mouse, especially from birth up through the nursing period. A further study of the literature also has been undertaken, especially of some early work which has been cited by very few recent authors or which had lost support either because of lack of confirmation or because of the apparent inconsistency of the conclusions with generally accepted biological principles. Furthermore, we felt that the possible sources of error in our interpretation of findings, in a tissue which has been so accessible to study for so long a period of time, needed further examination, both through study of more material and additional investigation of the literature on intestinal epithelium, reproduction in epithelial tissues, and the relationship of lymphocytes to epithelium.

In the earlier paper on this subject (Andrew and Andrew, '45) we cited Loreti ('35) as affirming an intracellular position of lymphocytes in the epithelium of the kidney of teleost fishes and also as probably true in the intestinal epithelium of some lower vertebrates. We have found that in the writings of some of the very earliest authors a similar view was expressed for intestinal epithelium. Thus Schaffer ('36, p. 92) says: "Eberth has been the first to see lymphocytes penetrate between and, as he thought, even within the cells of intestinal epithelium, an observation which was supported by Eimer (1867) and Arnstein (1867)." Paneth (1888) believed that lymphocytes did occupy an intracellular position.

In our own study of the early authors, we find that Eberth (1864) studied a number of mammals, including rabbit, cat, pig, and man, as well as lower vertebrates. Where round cells had fallen out of the epithelium he found sharply circumscribed spaces remaining.

Von Arnstein (1867) describes rounded or oval, more or less granulated cells partly between and partly in the cylinder cells and goblet cells in the rabbit, guinea pig, dog, and cat.

Paneth (1888) says that the lymphocytes, particularly in the mouse, are seldom seen near the free surface of the epithelial cells and doubts that they ever wander out into the lumen.

Beguin ('04) studying differences in the epithelium of the intestine of toads and lizards during digestion and in a fasting condition, found many more leukocytes in the epithelium of fasting animals. In animals during digestion he found them relatively scarce and seldom succeeding in even passing as far toward the lumen as the level of the epithelial nuclei. But he says of the fasting animals (p. 422): "The epithelium . . . encloses numerous migrating elements which infiltrate between the cells or even traverse them." Of the period of digestion he says (p. 444): "It seems that at the time when the cylindrical cell has the task of recovering food materials brought into the intestinal cavity, it repulses the leukocytes which are found in its interior or on its sides." Thus Beguin definitely thought the migrating leukocytes in toads and lizards to occupy, at least in many cases, a position within the epithelial cell.

Schaffer ('36), however, after examining the evidences, says (p. 92): "Studying surface sections of the intestine — and only such are decisive in this question, since in longitudinal sections the dim cell boundaries may easily lead to deception — I (1891) never could be persuaded of an actual intracellular position of a leukocyte in an epithelial cell. However, I often saw them lying in deep niches of one or two neighboring cells."

Certain authors have felt that there definitely are two nuclei of unlike type present within many of the epithelial cells of

the intestine and that their presence represents an important feature of the life history of the epithelial nucleus. The hypotheses to which this type of idea gave rise appear so opposed to our modern ideas of cell relationship and lineage that they receive scant attention at the present time. Nevertheless, the recognition by these authors of an apparently different type of nucleus, in addition to the characteristic epithelial nucleus, within an epithelial cell, as well as their attempts to explain the significance of the phenomenon, are well worthy of our attention when we ourselves are advocating the intracellular position of the lymphocytes.

Davydoff (1887) studying especially the epithelium over the follicles of the vermiform process in the guinea pig and the villi in the jejunum of a healthy, 22-year-old executed man, found leucocyte-like cells within the epithelial cells. He speaks of the dark nuclei of the leucocytes as "secondary nuclei" and suggests that they actually are produced by amitotic division of the "primary" clear epithelial nuclei. The newly formed leucocytes then wander out of the epithelial cells into the underlying stroma, according to his conception.

Grunhagen (1887) believed that binucleate cells might arise even on the summit of a villus, by mitosis of the original nucleus.

Guieysse-Pellissier ('12b) describes the phenomenon of lymphocyte migration into intestinal epithelium as a form of the process of caryoanabiosis (incorporation of nuclei of cells into cytoplasm of other cells with a change, often in the direction of rejuvenation, of the first-named nuclei) first described and named by him as occurring in the formation of foreign-body giant cells. In the production of such cells he found polymorphonuclear leukocytes engulfed in the young giant cells, the cytoplasm of the leukocytes disappearing, and the nuclei changing from the multilobed, pycnotic type to a typical rounded, vesicular giant-cell nucleus. This change in type of nucleus and "graft" of nuclei of one class of cell into the cytoplasm of another, he thought is seen also in the case of the lymphocytes which penetrate into epithelial cells. The

process here is visualized as one of replacement of the "old" epithelial nucleus which degenerates and disappears, probably, he says, by a process of diminution in size and eventually of solution in the cytoplasm.

He says definitely (p. 39): "I believe that, in a normal intestine, the leucocytes do not pass out into the intestinal cavity." Such passage would not have much purpose, of course, if the destiny of the wandering cells were chiefly to furnish new nuclei for the epithelium.

In more recent times the view of Guieysse-Pellissier has found some support in the results of Goldner ('29). This author found in rats after massive desquamation of intestinal epithelium caused by administration of ricinusol some phases of the regenerative phenomena which seemed to support the concept of caryoanabiosis. In some places where epithelium remained preserved there were both mitoses and amitoses, syncytial masses, fragmentation of nuclei, and penetration of "mesenchymal wandering cells into the epithelial cytoplasm, with apparent incorporation of the nuclei of these cells to serve as epithelial nuclei." Goldner says, however, (p. 95): "But until more ample information is obtained, this mechanism should be considered with all the reserve which is due to an hypothesis which for the present is so contrary to our existing knowledge of nuclear physiology."

No more recent authors, so far as we have found, have supported the hypothesis of caryoanabiosis in intestinal epithelium. Nor has the question, far more important in regard to our own conclusions, of the intracellular location or division of lymphocytes, been treated in recent times.

MATERIALS AND METHODS

In the present investigation white mice from the colony of the Institute for Investigation of the Biological Sciences were used. Preparations from twenty-three animals were made: two of 2 days of age, two of 4 days, two of 5 days, one of 6 days, two of 8, two of 13, two of 17, two of 18, two of 20, and two of 30 days respectively, and four adult animals. The

animals of ages through 20 days were subsisting on the milk of the mothers.

All of the mice were killed by rapid decapitation and the duodenum removed and fixed immediately with no attempt at washing out of its contents. With the exception of the two animals of 17 days the duodena were well filled with food material (milk or commercial feed as the case might be). The mother of the two animals in question was found dead at the time of their sacrifice and their duodena were empty or practically so, but of course the duration of this condition was not known exactly.

The material from the younger animals and from two of the adult mice (pieces of duodenum of 5 to 10 mm in length) was treated with the uranium-silver method of Cajal, as follows:

I. Fixation, from ½ to 1 hour (time that we have found optimum) at room temperature, in:

Formalin	15 cm³
Distilled water	85 cm³
Uranium nitrate	1 gm

II. Rapid immersion (three times) in distilled water.

III. Impregnation for 36 hours, at room temperature, in dark bottles, in aqueous solution of silver nitrate, 2.5%.

IV. Rapid immersion (three times) in distilled water.

V. Fractional reduction in the following manner:

(a) Prepare "solution A"

Formalin	15 cm³
Distilled water	85 cm³
Pyrogallic acid	1 gm

(b) Prepare "solution B"

Solution A	1	aa
Distilled H_2O	1	

(c) Reduce the pieces, for 20 minutes, in:

Solution B	1 volume
Acetone	5%

(d) Reduce for 24 hours in:

Solution A	1 volume
Acetone	1%

VI. Wash, cut frozen sections at 5 to 10 μ, dehydrate in alcohol, clear in pure creosote, and mount in Canada balsam.

With this method, clear images of the Golgi apparatus were obtained in the crypts as well as in the villi, although the relative degree of impregnation was not the same in the two locations. The results most favorable for the crypts were, at times, much less so for the villi, and vice versa. Some sections of the impregnated tissue were lightly stained with Carazzi's hematoxylin and with eosin, to ascertain better the location and relationships of the nuclei.

The duodena of the other animals (two adult mice) were fixed in 95% alcohol and treated with the method of Feulgen-Rossenbeck for the demonstration of the chromatin. Sections were cut on the freezing microtome at 5 to 10 μ.

We wish to express our sincere appreciation to the Phototechnical Laboratory of the Institute for Experimental Hygiene for valuable aid in the production of the photomicrographs.

OBSERVATIONS

We shall describe first in a general way the conditions found in the mice of different ages in regard particularly to the presence and relative amount of lymphocyte penetration of the epithelium and then pass on to a more detailed study of the exact position of the lymphocytes and of the changes which they undergo.

In the animals of two days the villi vary considerably in height, some being long and finger-like, others mere rounded prominences, like low hillocks protruding into the lumen. The crypts also are in a very rudimentary condition, often consisting of no more than a dozen cells as seen in a complete section of an individual crypt from mouth to fundus. Nevertheless, the distinction between the cells of the villi and those of the crypts is very sharp even at this early stage. The cell membranes are much more definitely seen in the villus and the cytoplasm of the cells here is far clearer. In the crypts the cytoplasm is relatively dark, cell membranes often almost impossible to distinguish, and at this stage, the nuclei are relatively long and narrow, presenting a crowded appearance

in comparison with the well spaced, relatively plump ovoid nuclei of the villi.

Another very definite distinction between the cells of the villi and of the crypts lies in the structure of the Golgi apparatus. In the cells of the crypts this appears as a relatively small, compact, closed network which while often apical, also occurs in numerous cases somewhat displaced to one side of the nucleus. In the cells of the villus the Golgi apparatus is larger and more complicated, some with very distinct long threads, sometimes with loops, but always appearing more open than does that of the crypts (see figs. 14 and 15). In the villus the Golgi apparatus also is more definitely oriented, appearing always, in our experience, at the apical pole of the nucleus.

So definite is the distinction between crypt and villus cells even at this early age when the crypts are beginning to form that there are places where we may see only two cells together on the surface which can be described as cells of the crypt type while the cells on either side are those of the general surface or villus type.

In these very young animals we see almost no lymphocyte migration into the epithelium of the villi. When it occurs, it is usually at the base and very seldom farther out on the villus. In the crypts, on the other hand, such migration, while scanty, is definitely more active.

In the animals of 4 through 13 days we find with the further development of the crypts an apparent general relative increase of lymphocyte migration into their epithelium, but the amount of such migration still appears definitely less than in the adult animals previously studied. The villi in animals of these early ages remain almost free of lymphocyte migration, so that we seldom see more than one or two lymphocytes in the epithelium in an entire cross-section.

In animals of 17, 18, and 20 days the lymphocytes in the crypts are much more numerous and many stages in their transformations within the epithelium are seen. Lymphocytes in the epithelium of the villi still are encountered only

rarely, but somewhat more often than in the younger specimens.

In mice of 30 days the crypts are the scene of a very intensive lymphocyte migration and the villi show far more of the process than at an earlier age, there being lymphocytes intercalated between the bases of many epithelial cells of the villi and not infrequently seen between the nuclei of epithelial cells and the striated border. Vacuoles of large size enclosing the lymphocytes both in the crypts and in the villi are conspicuous.

The sections from adult animals resemble in most respects those of the 30-day animals, having as much or more lymphocyte content and being similar in this respect to the intestines of adult black mice described in an earlier communication.

In the mice of all ages examined in the present work, the lymphocytes within the epithelium show definite changes. Most conspicuous of these is the increase in size, those on the apical side of the epithelial nuclei often appearing twice the diameter of those on the basal side. Accompanying this change in size is an alteration of the basophilic content of the nuclei such that the entire nucleus appears like an almost solid mass of darkly-staining material or contains large individual masses of such material. A frequent finding is the nucleus which has this material situated peripherally to form a complete or partial ring of deeply staining substance just beneath the nuclear membrane. The basophilic material also in many cases appears to be well impregnated with silver (see figs. 9, 10, 11), and from studies up to the present with the Feulgen method would seem to be largely true chromatin.

It is of great interest that the Golgi apparatus of the lymphocytes is retained and in fact seems to increase in size with the nucleus up to a certain point. In the enlarged lymphocytes apical to the epithelial nuclei it frequently is seen with great clarity and appears quite normal (see figs. 1 and 2). As described by Cowdry ('21) using the uranium-silver method, and by Estable ('30) in detail, using a variant of this

method, it is a relatively simple, well orientated type of Golgi network.

We should record, however, that Maximow ('28) obtained pictures of the Golgi apparatus of lymphocytes as "small black bodies . . . in the neighborhood of the centrioles" — an appearance almost certainly due to incomplete impregnation — and that the apparatus also was studied in more detail by Ehrlich ('34) whose description of the morphological characteristics for distinguishing lymphocytes from monocytes and other mononuclear cells are discussed by Bloom ('38) in relation to the general conclusions of Owens and Bensley ('29) on the intimate process of the osmic and silver techniques. The studies of one of us (Sosa) carried out in the laboratory of Professor Bensley ('43–'44) confirm us in the idea that, even if the reduction of silver in the cells does not have in it a chemical specificity, and if the silver method of Cajal is essentially morphological and not chemical, there does exist a true specificity of results which comes from the use of a selective fixative employed for a specific time. On the other hand, without disregarding the value of the osmic acid technique, it is interesting to recall the words of Cowdry ('21) who found, as is recounted in many other places, that "Cajal's method gives more definite results."

In this work, as in the previous study, many of the lymphocytes in the apical end of the epithelium are disintegrating. The modifications undergone by the Golgi apparatus (see figs. 1 and 2) are of such a nature that they can be interpreted as constituting a true reactional process consisting of certain well defined fundamental steps, that is to say: (a) a progressive phase and (b) a regressive phase.

In the first, the reticulum is found extended, with the trabeculae, and especially the enlargements of the net, in evident increase of volume to the point of appearing as a hypertrophied Golgi apparatus. Such appearances are seen, especially for the apparently swollen lymphocytes. Whether these really are swollen by an imbibition of fluid, or whether we are dealing here with a true hypertrophy, constitutes a

problem difficult of solution. Nevertheless, the greater complexity of the Golgi net along with its increase in volume tends to make us consider the second hypothesis more probable. Indeed, the simple swelling would explain the increase of volume of the enlargements and trabeculae of the net, but it could not explain the multiplication of such elements and the consequently great complexity of the Golgi apparatus.

In the second phase, parallel to the diminution in size of the lymphocyte, the reticulum is seen in an evident regressive atrophy, with an appreciable simplification of the network, already simple in its nature, as we recall from the description above. Further, different steps in golgiorrhexis can be made out, with the formation of golgiosomes, and final golgiolysis.

We feel no reasonable doubt as a result of the studies made earlier that the majority of lymphocytes in duodenal epithelium are in a truly intracellular location. Nevertheless, it has been of great interest to confirm this fact with the demonstration of Golgi apparatus both of the epithelial cell and of the intracellular lymphocyte. The vacuole about the lymphocyte frequently appears to press upon the epithelial nucleus so as to deform it. Such deformation varies all the way from a slight indentation or concavity of the nuclear membrane to an actual flattening of the entire epithelial nucleus in some extreme cases. The images obtained with the uranium-silver method (figs. 1, 6, 9, 10, 11, 16) do not leave room for doubt as to the position of the lymphocyte in the interior of an intracellular vacuole, the Golgi apparatus of the epithelial cell frequently appearing in a relation of proximity, and even contiguity, with the vacuole.

Pictures of extrusion of lymphocytes both into the lumen of the crypts of Lieberkuhn or from the villus into the lumen of the intestine are common. However, it may well be that many lymphocytes remain for some time (at present we have no way of saying for just how long) within the epithelial cells. The retention of a Golgi apparatus and the increase in size of it and of the nucleus argue for such a stay within the cell. Whether this is an actual stationary position for some

time or is a matter of a very slow passage through the epithelium is a problem for future solution.

The idea that many of the lymphocytes also undergo a more or less complete degeneration within the epithelial cells is borne out in all of these specimens. This process occurs by fragmentation with apparent solution of the fragments or by an enormous swelling and vacuolation. The latter process, while seemingly lacking entirely in some intestines in this study, is present in abundance in others. It gives rise to pictures confusingly like those of the goblet-cell, as described and pictured in the earlier work (Andrew and Andrew, '45). In cases of such degeneration within the cells the substance of the lymphocytes passes into the lumen in a form which would not be recognizable in a smear or wet preparation of intestinal contents and which is hard to recognize in histological preparations except where the actual process of extrusion fortunately is caught.

The presence in great numbers of mitotic figures of lymphocytes within epithelial cells was a striking feature of the findings reported in the black mice of Strain C57. In the present series of white mice this feature is quite as conspicuous in all of the animals from 17 days to and including the adults. In animals younger than 17 days, such mitotic figures are found in fair numbers on every section but appear fewer than in older animals. This difference, however, probably merely is correlated with the lesser degree of lymphocytic infiltration in the younger animals.

Our interest in these mitoses, as described in the earlier paper by one of us, was called forth by: the presence of an epithelial nucleus and a mitotic figure in the interior of the same cell; by the clearly intracellular position of many of the lymphocytes; and by the existence of well defined intermediate steps between the lymphocytes "in repose" and the mitotic figures. All of these reasons operate toward the same conclusion in the present material. The last-named, however, it seems to us, requires further emphasis.

A peculiar feature of the mitoses is the absence of figures similar to those described as characteristic of the prophase, with formation of the closed and open spireme. Such figures simply appear to be lacking. In their stead are peculiar crumpled or angular forms, frequently with a very deeply staining, basophilic (also argyrophilic, periphery) and a somewhat lighter center. Such a stage is seen in figure 3 in the cell B adjoining one in which a definite anaphase is present. The mitosis appears to continue from this step to the formation of chromosomes, passing on to metaphase and anaphase.

Aberrant mitoses with partial nuclear fragmentation are seen also in these animals. One of the most frequent aberrations consists in the retention of bridges of chromatic substance far into the anaphase, as seen in figure 6.

The curious fact of apparent disregard of cell boundaries in some of these mitoses, the mitotic figure extending into two or even three epithelial cells, apparently is a common phenomenon. This is the explanation, we believe, of the presence in many instances of two lymphocytes of very similar size and form, usually ovoid, in two adjoining epithelial cells. They represent recently divided daughter cells and a reestablishment of cell boundaries in the region of the epithelial cells in which the mitosis has occurred leaving the two new lymphocytic cells in separate epithelial cells.

It must be admitted, however, that the problem of whether an individual mitosis represents that of a lymphocyte or of an epithelial nucleus, especially when we consider that the epithelial cells are rapidly increasing in number by some form of reproduction, in these young animals, is not always easy to answer. The difficulties in this regard and further consideration of our reasons for considering the great majority of mitoses even in young animals as lymphocytic will form a part of our discussion.

Finally, we should mention the fact that, in our material, polymorphonuclear leucocytes are observable, although in smaller quantity than the lymphocytes, in distinct stages of migration across the duodenal epithelium. Many images of

this type are comparable in every way with those of other authors, especially, of Weill ('19 and '20).

As is known from the classical writings (Reinke, 1891; Meves, 1891, 1896; von Kostanecki, 1892; Henneguy, 1896; Jolly, 1900, '23; etc.) the polymorphonuclears of the rodents, as also other cells of different animals, frequently show the nucleus perforated, or of a more or less annular form (A, B, C, of fig. 7). We find it of interest to mention the old and beautiful observations of von Kostanecki and of Meves on the genesis of the annular nuclei. They demonstrated, as confirmed by Reinke, that the nuclei are formed in cariodieresis, and added that the opening in the nucleus, when it exists, is due to the late disappearance of the achromatic spindle and the formation of the carioteca in the interior of the crown formed by the chromosomes of the daughter cells.

Figure 7 shows various polymorphonuclear eosinophils, subepithelial in position, from the duodenal mucosa of a mouse of 9 days. Whatever may be the form of the nucleus, all of them show a Golgi apparatus located in the concavity of the nucleus. The apparatus is very small, and although at times it appears simple (G, H, of the figure) it constitutes, more frequently, a fairly complex network, made up of fine trabeculae and enlargements connected among themselves. In the conditions of our material of study, there was very rarely observed any fragmentation of these with liberation of Golgi bodies or, better, golgiosomes, to employ the nomenclature of one of us (Sosa).

DISCUSSION

We feel that the present study has amply confirmed the fact of the intracellular position of lymphocytes in intestinal epithelium. It has shown also that such a phenomenon begins very early in the life history, being present in the youngest animals studied, those only 2 days of age. The series of changes consisting in increase of size with pycnosis and, frequently, fragmentation, or extrusion of the whole lymphocyte into the lumen, is the same here as in the Strain C57. Also

the phenomenon of great swelling with vacuolation and extrusion is seen in many instances, but the significance of the difference in the two types of degeneration has not been established as yet.

In our opinion the most puzzling matter and the one most subject to an error of interpretation in this study is that of the nature of the mitoses occurring, whether they are those of lymphocytes or those of epithelial cells. The fact which makes the solution of this problem particularly difficult is that migration of nuclei of columnar epithelial cells during the interkinetic stage, with karyokinesis in the apical portion, has been described with considerable precision by various authors.

Thus Sauer ('36) says that in all classes of vertebrates in the epithelium of the lens, the otic vesicle, nasal pit, somites, and in the gut and its derivatives this process occurs. This author describes in detail the division of cells of the foregut of garter snake embryos. The cytoplasm of the "columnar" cell draws away from the lumen and in metaphase the cell is almost completely rounded. The internal third of the tube wall is free from nuclei except those in or near mitosis.

The same author had described a migration of nuclei in the neural tube, pointing out that the "germinal cells" near the lumen of the tube actually are the same cells as the columnar epithelial "spongioblasts." After the interkinetic nuclei of the latter have migrated to the lumen, the cytoplasm rounds up and the cell undergoes mitosis in this position, with a resumption then of the columnar shape and a migration of the nucleus away from the lumen.

Cohen and Berrill ('37), in describing post-embryonic growth and cell-division in various tissues, describe a very similar process in the gut epithelium of the frog tadpole, the young trout and the larva of the lamprey. In all of these cases there is migration of nuclei to the lumen, rounding up of cells, division, and a return of the epithelial cell to its former shape and position. The drawings presented resemble very much some of our pictures of mitoses and the inference might

be drawn that many of our mitoses of lymphocytes are really those of epithelial cells.

We do not believe that these appearances, however, are in any way sufficient to justify such a conclusion. As we have pointed out in the description of the mitoses, the spireme stages, or the stages of the prophase are not seen in our material and no seemingly possible intermediate stages appear to occur between the "resting" or ordinary epithelial nucleus and the clear-cut later stages in mitosis. On the other hand, such intermediate stages do exist between the pycnotic lymphocytic nuclei and these stages in mitosis (see fig. 3).

The products of mitosis appear to be small lymphocytes, situated far apically in epithelial cells and, in our opinion, subject to the degenerative changes seen among the lymphocytes in general in this location.

The presence of various types of aberrant mitosis seems to point to the lymphocyte as the cell undergoing division rather than to the epithelial cell, as we do not see the nucleus of the latter showing any particular degenerative changes.

The question of relative size of the mitotic figures is not of as much worth as might be thought "prima facie" in determining the type of cell undergoing karyokinesis. It would seem at least possible that relatively very small nuclei formed in mitosis might regain the size of epithelial nuclei. On the other hand, the relatively large size of some mitotic figures (see figs. 3, 4, 6, 10) does not argue, as might be thought, in favor of their being epithelial, as many of the lymphocyte nuclei in the apices of epithelial cells would have attained a very considerable size before mitosis.

One of the facts, relatively small in itself, but which we believe to be of considerable significance in the determination of this question, is the presence of certain mitotic figures within the epithelium in a basal position where there is no question of confusion of such figures with "migrating" nuclei of epithelial cells which are described as proceeding to the apical part of the cell. Such a mitotic figure is seen in figure 17. Here the mitosis is occurring clearly basal to the line of

epithelial nuclei and on the same level as many of the normal appearing lymphocytes seen so frequently at the base of epithelial cells. This position for the mitotic division of lymphocytes is relatively rare in comparison with the supranuclear position, but its occurrence here seems to us to be an indication of a beginning tendency to divide, even on entering the epithelium.

The question as to whether any mitosis of lymphocytes occurs in the epithelial layer has met with conflicting answers. Schaffer (1891) found lymphocytes in mitosis in man in the intestinal epithelium (but not, in his opinion, within epithelial cells, as we have seen in the introduction). Nicolas (1887) had seen mitoses of lymphocytes in the epithelial layer in the frog. Weill ('20) asserts that the lymphocytes between the epithelial cells show no longer any mitoses. It seems obvious that if many authors have noted the great numbers of mitoses in the crypts in recent times, they must have been dismissed as those of epithelial cells.

The question of mode of reproduction of the epithelial cells, if not entirely or in the main by mitosis, remains somewhat obscure. There are many figures in the crypts which could be interpreted as amitosis and occasional pairs of very similar nuclei in adjoining cells which might represent products of such division, but the problem requires further study.

In an interesting paper on the lymphocytes in the epithelium of the palatine tonsil, Kingsbury ('44) points out that the lymphocytes change during their passage in such a way that their nuclei tend to become polymorphous. This he interprets as an incipient fragmentation and degeneration. He also describes a variant mode of degeneration in which the chromatin concentrates next to the nuclear membrane, exactly as seen by us in the intestine. In such cases fragmentation may follow. This type of degeneration, he says, also occurs within the node, leading to formation of the "stainable bodies" which are phagocytized in the clear center.

Considering the widespread occurrence of lymphocyte migration through the epithelium in various parts of the body,

it seems surprising that the nature of the process in the intestinal tract and the changes occurring in the lymphocytes have not received more attention.

Kingsbury points out that many authors doubt that there is an active migration of lymphocytes through the epithelium onto the free surface. He says (p. 234): "The lymphocytes within the intestinal epithelium are usually inside the zone of epithelial nuclei and have not been noted traversing the striated cuticular border; it has even been suggested that they may return to the underlying lymphatic tissue stroma (Kindred, '42).

While Bunting and Huston ('21) showed that lymphocytes are found to some extent on the inner surface of the intestine, Kingsbury says that these may be adequately accounted for as liberated through epithelial defects, many of them doubtless caused by manipulation in preparation or by normal loss of epithelial cells during life.

Guieysse-Pellissier had taken the stand that in the normal intestine lymphocytes probably do not pass through the epithelium into the lumen. More recently Hellman ('34) has suggested that these cells remain in the epithelium as a defensive barrier.

In our opinion, the extensive work of Jassinowsky ('25a and b) in this regard scarcely can be refuted. By the method of repeated irrigation with warm physiological salt solution and later counting of elements and making of smears from this solution he found an emigration of polymorphonuclears into the mouth cavity and an emigration of lymphocytes into the lumen of the intestine, varying in degree in different portions but extending through its entire length.

We believe that we have demonstrated amply in a histological sense the extrusion of lymphocytes from epithelial cells into the lumen, both by way of the crypts and the villi. The process may be described as an "active" one in the sense of many lymphocytes being extruded. On the other hand, we feel that it is a "passive" one in the sense that the final act of extrusion is usually due to factors other than the ameboid

motion of the cell which almost undoubtedly did operate in the penetration of the epithelium. Further, the extrusion is, in most cases, an extrusion of altered lymphocytes not infrequently unrecognizable as such.

SUMMARY AND CONCLUSIONS

1. The position of lymphocytes within the cells of intestinal epithelium, both in the crypts and in the villi of the duodenum, has been confirmed in a strain of white mice.

2. The changes undergone by the lymphocytes, increase in size, pycnosis, fragmentation, or at times great swelling and vacuolation, are seen in these animals as in the strain of C57 black mice.

3. The demonstration of the Golgi apparatus shows this element well-conserved in many intracellular lymphocytes even after the nucleus of the latter has become large and pycnotic. Topographic relations of the Golgi apparatus of the epithelial cell to the lymphocyte in its vacuole add further evidence for the intracellular situation of the lymphocyte.

4. Mitoses of the lymphocytes occur in the epithelial layer in great numbers. These are nearly always in the cells apical to the epithelial nuclei, but we have found cases where lymphocytes divide before reaching the level of the epithelial nuclei. Mitoses are often aberrant.

5. Age differences in the white mice studied (range in age, 2 days to adult) are definite and consist in an increase in the amount of lymphocytic infiltration from the earliest age up to the 30-day stage. Even in the youngest animals, however, the crypts are the site of some penetration.

6. The ultimate fate of the majority of the lymphocytes seems to be extrusion into the lumen of the intestine, either as whole cells or in a form so altered as to be unrecognizable.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

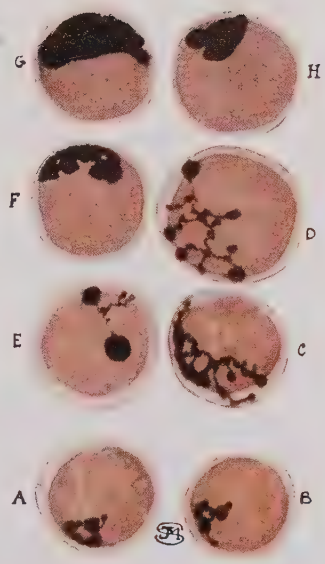
The magnification of all figures in plates 1 and 2 is approximately 4000 ×.

1 Cells from near the base of a villus in the duodenum of a mouse 4 days old. The intracellular position of the lymphocyte in the cell in the center is seen. The lymphocyte is much larger than those found at the bases of epithelial cells and appears surrounded by a vacuole, and its Golgi apparatus appears hypertrophied. There is a definite though not marked difference in appearance between the epithelial cell in which the lymphocyte lies and those adjoining it. Cajal method for Golgi apparatus, with hematoxylin stain for nuclei.

2 Different stages in the transformation of the intracellular lymphocytes. The variations in the Golgi apparatus are seen: normal, A. B, hypertrophied, C, D, and agglutinated, E, F, G, and H, before the final stages of golgiorrehexis and golgiolysis, in turn prior to the fragmentation and distintegration of the lymphocytes. Mouse of 4 days. Uranium-silver method of Cajal. Nuclear coloration with hematoxylin.

3 Lymphocyte mitoses in a crypt of Lieberkühn. In the large vacuolar space A is seen an advanced anaphase while the smaller vacuole, B contains a nucleus which we consider to be in the initial stage of mitosis of these cells, with a concentration of chromatin against the nuclear membrane. Hematoxylin-eosin.

1



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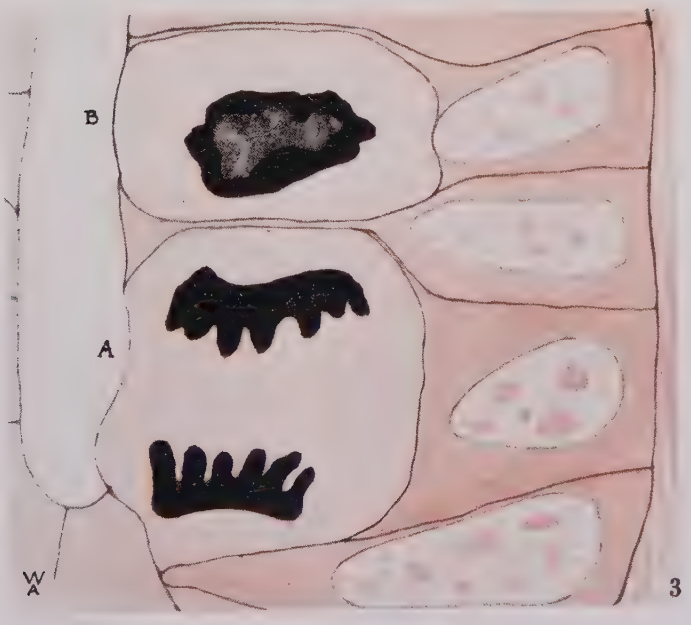

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PLATE 2

EXPLANATION OF FIGURES

4 Mitosis of a lymphocyte above the nuclei of two epithelial cells in a crypt of Lieberkühn of a 20-day-old mouse. The considerable difference in size of the epithelial nuclei, compared with those in adjoining cells, is of interest. Hematoxylin.

5 Telophase nuclei of lymphocytes lying side by side in apical ends of epithelial cells of a crypt in a 20-day-old mouse. Uranium-silver method of Cajal plus hematoxylin.

6 Mitosis in a crypt in which it is somewhat difficult to distinguish the epithelial and lymphocyte nuclei. The latter, however, usually are smaller, darker, and surrounded by a vacuole. The anaphase in question shows the retention of a bridge of chromatin between the two groups of chromosomes and is an example of the frequently occurring aberrant mitosis. Hematoxylin.

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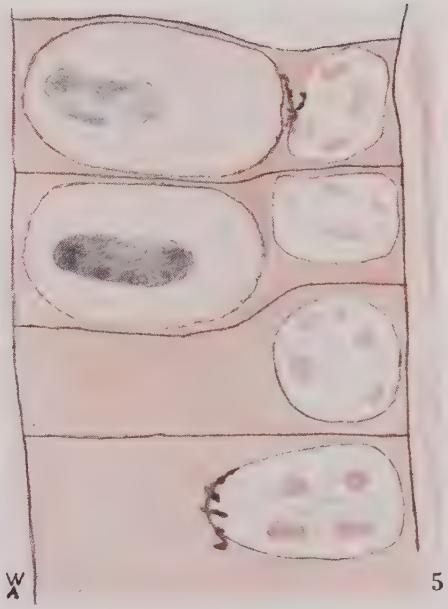

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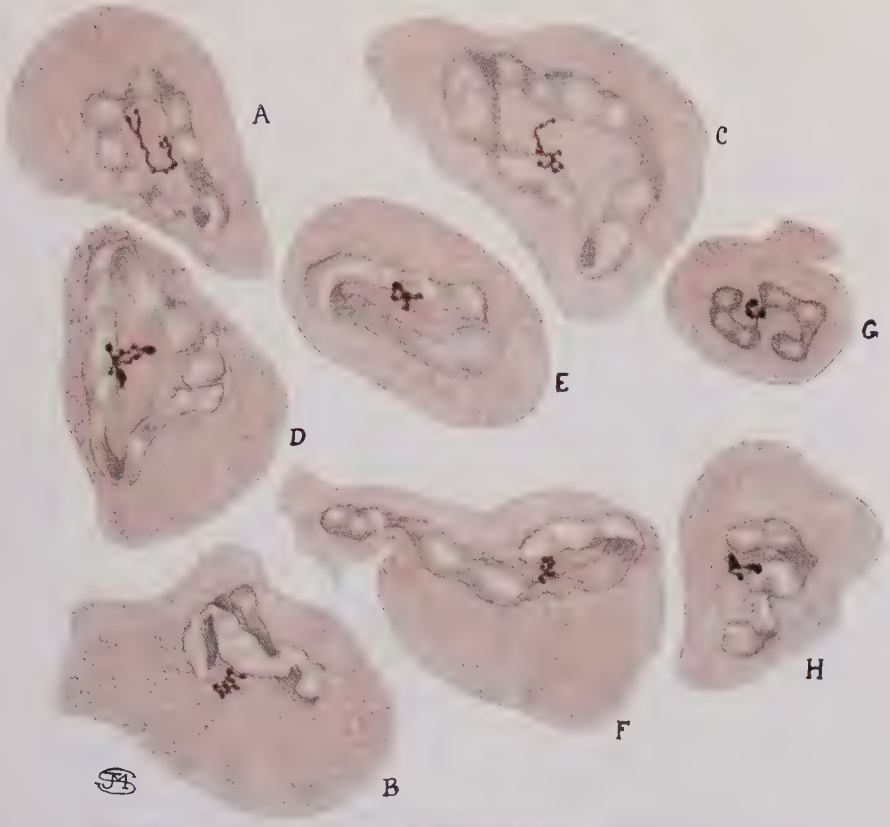
PLATE 3

EXPLANATION OF FIGURES

7 Polymorphonuclear leucocytes, probably eosinophiles (see the discussion in the text) from the connective tissue around the glands of Brunner, in a mouse of 30 days. The Golgi apparatus is small and relatively simple, but clearly reticular. Cajal method plus hematoxylin. × 4000.

8 Crypts of Lieberkühn of a 2-day mouse. The Golgi apparatus appears as a small, compact network, usually apical but sometimes to one side. There is very little infiltration of the epithelium by lymphocytes at this early age. Cajal method. × 650.



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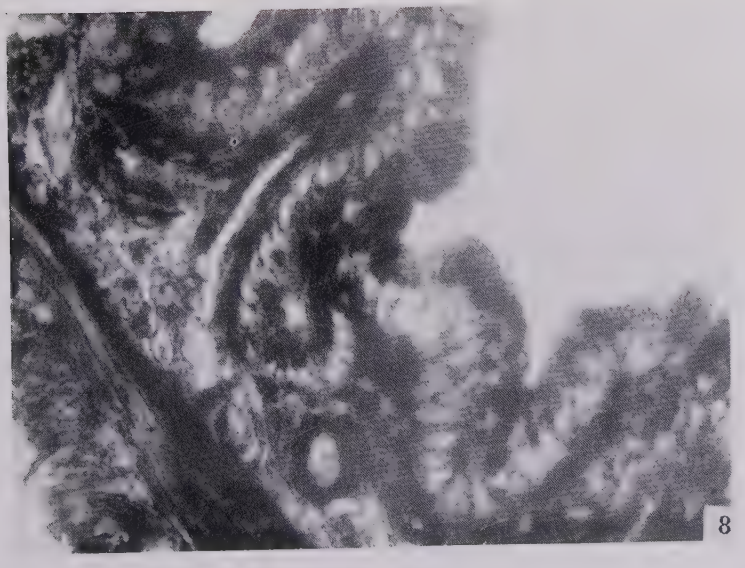

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PLATE 4

EXPLANATION OF FIGURES

9 Crypt in a mouse of 30 days. The large amount of infiltration with lymphocytes and the nuclear changes undergone by these are seen. Cajal. × 650.

10 Late anaphase (left) and probable prophase (right) of lymphocytes in crypt of a 20-day mouse. Hèmatoxylin. × 970.

11 Crypt from a 30-day animal. A very large vacuole containing an altered lymphocyte is seen actually protruding into the lumen. Two less severely altered lymphocytes are seen lower in the epithelium. × 970.

12 Crypt of a 30-day mouse. The very large vacuole, containing a lymphocyte, has altered the shape of the epithelial nucleus below it (just below center of field). Cajal method. × 650.

9
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11
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PLATE 5

EXPLANATION OF FIGURES

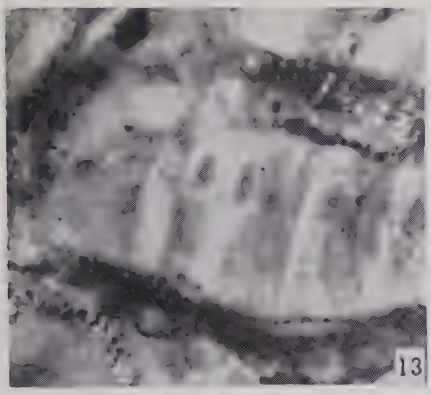
13 Two telophase nuclei of lymphocytes in the apical ends of epithelial cells in a crypt of a 20-day mouse. Hematoxylin. × 970.

14 Villus of a mouse of 4 days. The Golgi apparatus of the epithelial cells of the villi is in general larger and looser than is that in the cells of the crypts (compare with fig. 15). Lymphocytes are practically lacking from the epithelium of the villi and are rare in the crypts in these very young animals. × 970.

15 Crypt in a mouse of 30 days. The difference in the Golgi apparatus of the crypts (see especially the two cells on the right hand side of the figure) as compared with those of the villi, is seen. Epithelial nuclei are light-colored here, almost white in the figure. Note also the presence of lymphocyte migration, evidenced by the large intracellular lymphocyte which has completely distorted its epithelial surroundings. Cajal method. × 970.

16 Crypt of Lieberkühn of a 2-day-old mouse. The Golgi apparatus is relatively small and compact and lymphocytic infiltration is not seen in this field. Cajal method.

17 Portion of a villus of a 30-day mouse. A mitotic division of a lymphocyte (telophase) is seen and appears to extend across at least two epithelial cells. Such divisions below the layer of epithelial nuclei are relatively rare but seem to us important in aiding to verify the lymphocytic nature of the divisions above the row of nuclei and in showing that there is some tendency for lymphocytes to divide as soon as they enter the epithelium. Another lymphocyte is seen in the cell on the far left. Golgi apparatus not seen, but cell outlines well shown. Cajal method. ×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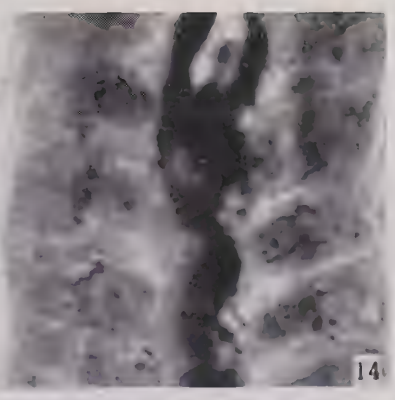
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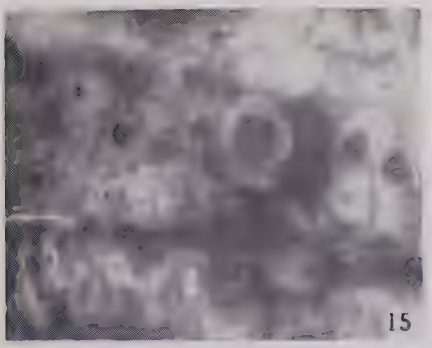
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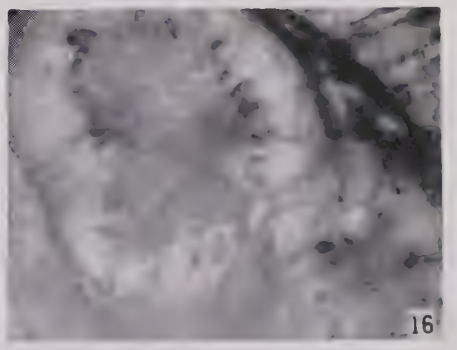
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17

PLATE 6

EXPLANATION OF FIGURES

18 Crypts of Lieberkühn of an adult mouse. The great degree of infiltration of the epithelium by lymphocytes is seen. In a number of the lymphocytes the chromatin is accumulated in dense masses in one side of the nucleus or peripherally close against the nuclear membrane. Feulgen-Rossenbeck method. × 650.

19 Crypts of Lieberkühn of an adult mouse. Several mitoses of lymphocytes are seen. The variation in orientation of the mitotic figures is evident. Feulgen-Rossenbeck method. × 650.

18

19

REACTION OF THE DIGOXIN-INJURED MYOCARDIUM OF RATS AND MICE TO VITAL DYES [1]

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NINE FIGURES

INTRODUCTION

Intracellular manifestations of injury and necrosis include alterations in total cellular, nuclear or cytoplasmic size; deviations in cytoplasmic and nuclear tinctorial reactions to various stains; differences in the pattern and structure of cytoplasmic granules and mitochondria; and the presence of atypical elements within given cells (i.e., droplets, granules, vacuoles, cytoplasmic and intranuclear bodies, etc.). Many necrotic cells are definitely smaller than normal, but enlargement of cells also represents a response to injury. Cloudy swelling, particularly of renal tubular epithelium, indicates a cellular reaction to damage that may be caused by a variety of agents. Epidermal epithelial cells frequently become very enlarged and vacuolated when infected by any of several viruses including those of varicella and variola.[3]

The definite nuclear changes in necrosis are fairly limited. The phenomena of pyknosis and karyorrhexis and the changes in nucleic acid which are responsible for them have been

[1] This study has been supported by the Medical Research Fund of the Graduate School of the University of Minnesota and by research funds of the Louisiana State University School of Medicine.

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[3] Excellent discussions of cellular responses to injury are presented by Forbus ('43) and Moore ('45).

reviewed by Moore ('45). Intranuclear inclusions constitute a response to injury and are usually associated with viral infections, but also may possibly result from other types of damage (Forbus, '43). Cowdry and Paletta ('41) have demonstrated changes in intranuclear viscosity in neoplastic and hyperplastic cells which may well be an expression of reaction to injury induced by percutaneously applied methylcholanthrene. Chromatolysis represents a response to various deleterious agents and conditions on the part of certain nuclear constituents of nerve cells. Changes in cell, nuclear and nucleolar size are also induced by carcinogenic hydocarbons (Cowdry and Paletta, '41a).

Atypical growth phenomenon may indicate injury of cells. Certain warts of man, the Rous sarcoma of chickens, and the Shope papilloma of rabbits are all examples of a proliferative cellular reaction to infection by specific viruses. Also, a type of nuclear "proliferation" occurs in another viral infection, varicella (see Forbus, '43, for a review). In some instances metaplasia and hyperplasia which may or may not "become" neoplastic represent concomitant or attendant responses to a carcinogenic hydrocarbon (Cramer and Stowell, '42; Cowdry, '43; Williams and Strong, '44; Howes, '46; Kirschbaum, Williams and Bittner, '46).

Histochemical methods allow in some instances fairly exact identification of the intracytoplasmic inclusions which occur in several diseases. Among these intracellular elements are glycogen, specific lipids, and several minerals. Atypical distribution of the so-called "fuchsin" pigments is of value in indicating cellular damage in conditions such as hemochromatosis. "Ceroid" pigments occur within many tissues during senility in man and lower animals and are also deposited in various cells of the latter as the result of certain dietary deficiencies (for review of literature see Mason and Emmel, '44 and '45; Moore, '45; Pappenheimer and Victor, '46).

The intracellular deposition of the various materials which have been mentioned might indicate atypical synthesis and retention of these substances, but another mechanism may be

that injured cells possess an impairment of the selective permeability of the cell wall. Several types of injury alter the reaction of various cells to parenterally administered dyes. This atypical "staining" has been observed to be either cytoplasmic or nuclear, or both. In other circumstances damaged cells exhibit a decreased ability to absorb and "concentrate" vital dyes (Oliver, '15; Oliver, Bloom and MacDowell, '41). The use of vital dyes to indicate cellular injury has been better demonstrated in relation to epithelial cells because they are injured with relative ease and such cells also offer fairly exact definition or delimitation as cellular units. To the contrary the precise structure of muscle (on the basis of cellular units) is not so well indicated in the usual histological preparations of this tissue. For these several reasons the experiments about to be described were initiated to study the reaction of damaged myocardium to parenterally administered vital dyes. A cardiac glycoside (digoxin) was used as the noxious agent with which to induce injury of this tissue.

MATERIALS AND METHODS

Twelve rats and 24 mice were used in the experiment. All animals were young adult males. The rats were stock albinos weighing from 225 to 250 gm each. The mice were of 2 inbred strains (Strong A and C3H) and weighed 22–25 gm each.[4] The rats and mice were free of any type of spontaneously occurring cardiac disease. Both strains of mice are subject to spontaneous mammary and other neoplasms but the animals in question were younger than the age at which such tumors occur. Therefore except for the experimentally produced cardiac lesions it seemed that all of the animals were in good health.

The dyes, trypan blue and chlorazol fast pink, were injected subcutaneously in 0.5% aqueous solutions. The digoxin solutions contained 0.25 mg of digoxin in each cm^3 of 35% alcohol.[5]

[4] The mice were from the colony of Dr. Arthur Kirschbaum. Dr. Kirschbaum's cooperation and generous assistance are gratefully acknowledged.

[5] The digoxin was supplied by Dr. John S. LaDue. The authors wish to thank Dr. LaDue, Dr. E. L. Burns and Dr. M. B. Visscher for advice and suggestions.

The plan of the experiment is shown in table 1 and needs no further discussion here. All animals were examined carefully at autopsy and tissues were fixed in a formol-alcohol-acetic acid solution (Williams and Hodge, '43). These tissues were dehydrated in dioxan, cleared in cedar oil and embedded in paraffin. Complete frontal sections of hearts were cut at 6 to 10 μ and mounted on glass slides. Two or more slides representing sections of hearts from each of the 36 animals were subjected to each of the following procedures: (1) paraffin removed from xylol and the sections covered as usual with clarite and glass cover slips; (2) stained with hematoxylin or toluidine blue; (3) stained with eosin or acid fuchsin; (4) stained with hematoxylin and eosin. The advantages of these various methods in the study of cells containing vital dyes have previously been discussed (Williams and Hodge, '43). Sections of hearts from the rats were also stained by the Mallory connective tissue method.

FINDINGS

Frequently the rats and mice became unconscious immediately after the injection of digoxin. They remained in such a condition for 10 to 20 minutes and during this period respiration was slow and shallow and the heart rate was diminished. Recovery from this state seemed complete within 30 minutes.

No abnormalities were observed at autopsy other than a slight amount of pulmonary edema in rats. There was no gross anatomical evidence of the subsequently described cardiac lesions.[6]

Deposition of dye in hearts of control animals

Numerous dye-containing cells were observed in the hearts of the rats and mice which had been injected with vital dyes but which had not received digoxin. All of these cells seemed to be true macrophages except the pericardial and epicardial

[6] Dr. B. J. Clawson's assistance in the study and interpretation of the histological material is greatly appreciated.

TABLE 1

NUMBER OF ANIMALS	EXPERIMENTAL PROCEDURE							FINDINGS (HEART)
	Treatment	Days						
		1st	2nd	3rd	4th	5th	6th	7th
5 albino rats [1]	Trypan blue [3] Digoxin [4]	0.5 cm³ 0	S 0	S 0	S 0	S 0	S 0	Killed	Dye limited to macrophages located for the most part in subepicardial and perivascular sites.
4 albino rats	Trypan blue [3] Digoxin [4]	0.5 cm³ 0.25 mg	S S	S S	S S	S S	S S	Killed	A few small myocardial lesions in 6 rats and much larger foci of injury in 1 rat. Dye was present in necrotic cardiac muscle fibres and in macrophages which were numerous in such areas. Dye was also in the so-called "Anitschkow myocytes."
3 albino rats	Chlorazol fast pink [3] Digoxin [4]	0.5 cm³ 0.25 mg	S S	S S	S S	S S	S S	Killed	
4 A strain mice [2]	Trypan blue [3] Digoxin [4]	0.1 cm³ 0.10 mg	S 0	S 0.05 mg	S 0.10 mg	S 0.10 mg		Killed	No definite myocardial lesions in 7 mice. A few damaged fibers in 1 mouse. In 1 mouse there were numerous small areas of myocardial damage and dye was in the injured fibers.
5 A strain mice	Chlorazol fast pink [3] Digoxin [4]	0.1 cm³ 0.10 mg	S 0	S 0.05 mg	S 0.10 mg	S 0.10 mg		Killed	
5 C3H mice	Received 0.1 cm³ of trypan blue [3] daily for 12 days and were then killed								Dye limited to the usual macrophages.
10 C3H mice	Received 0.1 cm³ chlorazol fast pink [3] daily for 12 days and were then killed.								

[1] The rats weighed approximately 200 gm each.
[2] The mice weighed from 22–25 gm each.
[3] 0.5% aqueous solutions injected subcutaneously.
[4] Digoxin (a glucose of *Digitalis lanata*) was injected subcutaneously to the mice and intraperitoneally to the rats. The alcoholic (35%) solution which was used contained 0.25 mg of Digoxin in 1 cm³. S: same treatment as on preceding day.

mesothelium. The majority of the dye-storing macrophages were located in perivascular or subepicardial sites. They were usually round in shape and contained large collections of dye (figs. 1 and 8). In general such phagocytic cells were more numerous in rats than in mice. Almost without exception those in the myocardium were large and round in shape. Such cells were frequent in atria and ventricles. A site of many dye-storing cells was the areolar tissue adjacent to the adventitia of the great vessels at the base of the heart. Such macrophages tended to be more spindle shaped than round and contained accumulations of dye which were smaller than those in the macrophages of the myocardium. In the non-digoxin-treated animals no dye was present in the cardiac muscle fibers, connective tissue fibers, or endothelial cells. Also, no dye was observed in smooth or skeletal muscle fibers of either control or digoxin-treated animals. It seems worthy of mention that the so-called "Anitschkow myocytes" which were observed in the rat hearts contained small granules of vital dye and this would seem to add further evidence that these cells are cardiac histiocytes (Anitschkow, '13; Ehrlich and Lapan, '39; Clawson, '41). Further, the mural elastic tissue of the large vessels at the base of the heart was lightly stained or colored by the vital dyes.

Deposition of vital dyes in damaged myocardial fibers and the histopathology of the lesions produced by digoxin

Since the histology of digitalis-induced myocardial lesions has been fully described in several accounts there is no need to present more than a summary here.

Damage to the heart by digoxin was limited to the myocardium. Quantitatively this was greater in rats than in mice (table 1). All of the rats showed some myocardial injury while such lesions were clearly indicated in only 2 of 9 mice (fig. 2). In both rats and mice the areas of damage were small, diffuse, and were distributed without any particular pattern throughout the ventricular myocardium.

The sections which had been deparaffinized with xylol and covered with clarite and cover slips clearly showed the increased amount of vital dye in the hearts of 9 of the 16 digoxin-treated animals (table 1, figs. 8 and 9). Distribution of dye in mesothelial cells and macrophages was the same as in control animals except for an increased number of the latter. In addition there were many small masses of dye arranged in fairly straight columns (figs. 4 and 9). The length of such accumulations was not great and the dye was deposited as small irregular particles or granules (figs. 3, 6, 9). Adjacent to these columnar particulate collections there were larger and darker clumps of dye. Study of stained (nuclear and/or cytoplasmic) sections demonstrated the particles arranged in columns to be dye located within damaged cardiac muscle fibers while the larger masses consisted of macrophages containing relatively greater amounts of the vital dye (figs. 3–6).

The morphological characteristics of the myocardial lesions may be summarized as follows (figs. 2–6, 9): (1) There was frequently a longitudinal splitting or rupture of the muscle fibers. (2) Such fibers stained poorly with eosin and there was absence of the usual striations. In addition there were small fuchsinophilic granules within the remaining cytoplasm. (3) In some instances there was a slight amount of hemorrhage and macrophages containing vital dye were numerous around the damaged portions of the muscle fibers. (4) Small granules or particles of vital dye were present within the cytoplasm of the injured fibers.

The myocardial lesions were limited to the ventricles and no intrafibral dye deposition was observed in the atria. Deposition of dye in cardiac muscle fibers was limited to those areas which showed other indications of injury both within and around the fiber. However, areas of myocardial damage were observed in which no vital dye was present in the fibers. In such cases it is possible that minute dispersions of dye had been lost by solution in aqueous reagents which were used in staining the sections with eosin, hematoxylin, etc.

In the digoxin-damaged eosin-stained sections of hearts from trypan blue-injected animals it was quite easy to locate the poorly stained portions of fibers which contained trypan blue. That is, the damaged portion of a fiber which contained trypan blue apparently possessed less affinity for eosin. For the study of animals which had received chlorazol fast pink it was more satisfactory to use sections stained with hematoxylin without a counterstain.

The vital dyes occurred in damaged fibers as particles or granules and the largest accumulations were smaller (figs. 2–6, 9) than those present in the macrophages (figs. 1, 7–8). The dye was frequently deposited peripherally within a given fiber (figs. 4, 5). Such dye was located within the remaining cytoplasm of the fiber and was not there as the result of being secondarily within macrophages which had entered the injured portion of a fiber. As would be expected, some dye-containing macrophages had entered such areas but it was easy to differentiate dye within such cells from that present actually within the substance of a fiber (figs. 2–6). The major criteria of differentiation under such circumstances were the nuclei of macrophages and the size and distribution of the dye particles.

The vital dyes located within injured areas of fibers seemed to be autonomous intrafibral deposits rather than to have combined with any cytoplasmic element and thus acted as a stain. Such granules or particles of dye showed no pattern of distribution which would indicate any morphological relationship to the surrounding cytoplasm (figs. 3, 4, 6).

Trypan blue was more in evidence within necrotic areas than was chlorazol fast pink. However, this is probably of no absolute significance. Tryan blue because of its color is more obvious than chlorazol fast pink. Also, the relative solubilities of the 2 dyes in the various solutions to which the tissues were subjected must be considered. Finally, it may have been that the more severely damaged myocardia were from animals which had received trypan blue or that the time interval

was optimal for maximum deposition of dye in necrotic myocardial areas.

The vital dyes "indicated" the digoxin-induced myocardial damage in 2 ways: (1) As would be expected, dye-containing macrophages were frequent in the sites of injury. (2) Particles and granules of vital dye were deposited in the remaining cytoplasm of injured portions of heart muscle fibers.

DISCUSSION

I. Intra vitam staining of injured cells

Vital dyes have frequently been used to study injured and necrotic epithelium of renal proximal convoluted tubules (Oliver, '15; Oliver, Bloom and MacDowell, '41; Williams and Hodge, '43). The interpretation of results in such studies is complicated by the normal function of the proximal convoluted tubules in the renal mechanisms which are responsible for the excretion and retention of such dye stuffs (Susuki, '12; Oliver, Bloom and MacDowell, '41). These epithelial cells when damaged apparently lose their ability to concentrate (intracellularly) such dyes and in addition there occurs a diffuse coloration of the cytoplasm with or without nuclear staining (Oliver, '15; Oliver, Bloom and MacDowell, '41).

Several other cells show atypical reactions to vital dyes. It has been observed that nerve cell nuclei exhibited intra vitam staining when damaged by trauma or by viral infections (MacCurdy and Evans, '12; Macklin and Macklin, '20; McClellan and Goodpasture, '23). Such dyes also occurred in atretic ova in rodents (Williams and Hodge, '43). Winternitz and his associates (Evans and Winternitz; Winternitz and Hirschfelder, '13; Kline and Winternitz, '15) described intra vitam staining of nuclei and cytoplasm of necrotic heterophilic leucocytes within exudates occurring in the lungs of rabbits with experimental pneumonia. These workers have suggested that failure of the absorbed dye to stain all of the granules of a single heterophil might indicate that atypical intracellular conditions rather than altered membrane per-

meability were responsible for the dye within the injured or necrotic leucocytes. However, Downey's demonstration ('17 and '18) that leucocytes store vital dyes when in extravascular sites might well explain the presence of dye granules within heterophils on the basis of a "normal" phagocytic mechanism rather than resulting from injury alone, whereas the nuclear staining would indicate injury or death.

Vital "staining" is probably an inaccurate term except as a description of the in vivo gross staining of tissues and organs by various dyes. The available histological methods do not allow the demonstration of whether true staining as well as intracellular deposition of dye granules occurs in such tissues. These dyes most probably pass through endothelial cells but the usual preparations give no clear and dependable demonstration of the dye within such cells (Downey, '17 and '18; Cappell, '29a and b; Zahl and Waters, '41). It is well demonstrated that these dyes normally not only enter macrophages and some of the epithelium of the nephron, but further, they increase in quantity or accumulate within such cells. Neither of these mechanisms is an example of what is ordinarily considered to be staining. In macrophages the storage of dye is thought to be similar to, if not identical with, phagocytosis and of what has been termed segregation. The epithelium of the proximal convoluted tubules (re)absorbs and "concentrates" most of these dyes (Susuki, '12; Oliver, Bloom and MacDowell, '41). Fat cells seem to be "stained" intra vitally by several water soluble dyes but again the term staining may be incorrect since the dye might occur within these cells as a result of a mechanism similar to phagocytosis (Dogliotti, '28; Goldner, '34a and b; Bremer, '38; Chang, '40; Williams, '42; Williams and Hodge, '43). The presence of dye within the epithelium of lactating mammary glands, serous membranes, and the choroid plexuses probably results from specialized abilities of these cells in relation to excretion, absorption, and diffusion, rather than being indicative of staining of cytoplasmic elements. The dye would seem to be a foreign body which possesses color. Therefore these dyes do

not truly stain the normal cells in which they occur since they do not combine with or precipitate on the surface of the cytoplasm and also do not dissolve in it as in the case of fat stains.

The intra vitam nuclear coloration which occurs in damaged and necrotic cells may represent true combination of the dye with nuclear material and therefore staining in the usual sense of the term. When dyes which in vitro are highly soluble in water do occur within a nucleus under such conditions they have apparently become much less soluble in water, and further, such vitally stained nuclei do not react to the usual basic dyes which would ordinarily stain nuclear material most readily (Williams and Hodge, '43). The previously mentioned tinting of elastic fibers by vital dyes (especially trypan blue) may also represent true staining.

Many dyes can be reduced to a colorless or leuco form and beginning with the historical work of Ehrlich in 1885 such stains have been used to measure or study oxidation and reduction in animal tissues (Bodansky, '34; Conn, '40). Of these dyes methylene blue is the only one which is in general use in histology and the azo dyes commonly employed for intra vitam staining have apparently not been utilized to any considerable degree in the type of study mentioned above. These azo dyes when deposited in such cells as macrophages and epithelium of the proximal convoluted tubules of the kidney usually have the same general color that they possess when in the aqueous solution used for parenteral injection. Therefore it has been assumed that these dyes occur intracellularly in their oxidized form. This may or may not be correct, but it seems likely that the usual histological preparations would only demonstrate dye in such a form. In the present study it was observed that a colorless leuco form of chlorazol fast pink resulted from treatment of this dye with formaldehyde and evacuation of oxygen. Subjection of trypan blue to the same procedure caused some loss of color but quantitatively much less than in the case of the other dye. Addition of NaOH without further treatment resulted in very striking

changes in color and subsequent heating produced loss of color by both dyes. Solutions of trypan blue thus treated contained a small amount of blue precipitate whereas the chlorazol fast was not only colorless but was also free of precipitate. These variations in response to in vitro reduction on the part of these 2 dyes might possibly have some relation to the observed superiority of trypan blue as an indicant of myocardial damage. However, in a previous study chlorazol fast pink was very satisfactory in demonstrating damage to the epithelium of the proximal convoluted tubules both as to cytoplasmic and nuclear staining (Williams and Hodge, '43). The addition of formaldehyde to aqueous solutions of both dyes caused no significant changes in the color of either. There are several reports which describe the in vitro and in vivo use of various dyes in studying oxidation — reduction potentials in a variety of living cells (Clark, '25; Needham and Needham, '25 and '26; Cannan, Cohen, and Clark, '26; Eadie, '28; Cohen, Chambers, and Reznikoff, '28; Visscher, '42).

II. Reaction of damaged myocardial fibers to vital dyes

The intra vitam deposition of granules and particles of dye within cardiac muscle fibers is in itself atypical. There is no evidence that parenterally administered dye occurs within normal muscle since such dye has not been observed in voluntary or involuntary muscle fibers in either a fixed or fresh state (Rous, '25; Cappell, '29b; Williams and Hodge, '43). The first explanation for the entrance of these dyes into damaged muscle fibers would seem to be based upon an alteration in the permeability of the cell wall. Also to be considered is the possible existence of a local alteration in the gradient of vascular permeability (Rous, Gilding and Smith, '30). This latter interpretation would assume that the injury produced by digoxin included sufficient local damage to capillaries to allow an increased diffusion of dye into the sites of myocardial damage. Thus a relatively greater amount of dye would be "available" for absorption by the damaged fibers or diffusion into them. Many studies of increased capillary

permeability and other vascular alterations which exist within sites of inflammation have been reviewed in considerable detail by Forbus, '43; Moore, '45; Menkin, '46. Some of these vascular alterations may have existed in the sites of myocardial injury produced by digoxin. Both increased permeability of the cell wall and of capillaries may have been active components of the mechanism which resulted in the presence of dye within the injured myocardial fibers.

Kiyono ('14) and Forbus ('26) have described the intrafibral deposition of dyes within skeletal muscle fibers which had been damaged by such agents as heat, alcohol, phenol, and as the result of ischemia. Forbus found that in animals which had received vital dyes during the period of muscle necrosis " . . . cells which are morphologically unquestionably of muscle origin are packed with the vital dye." Forbus discussing the previous work of Kiyono said, "Kiyono states that the regenerative muscle cells were unstained in his preparations which were obtained from experiments similar to ours, but was not sure but that some of the muscle cells might 'break loose from the sarcolema,' as he put it, and become phagocytic." These investigators induced more severe damage to skeletal muscle than was achieved in the myocardium in the present study. Concomitantly there apparently occurred a greater amount of intrafibral deposition of dye. However, a qualitatively similar response on the part of damaged muscle fibers existed in their experiments with skeletal muscle and the present one concerned with cardiac muscle.

As described earlier the dyes were in damaged myocardial fibers in the form of small granules or particles. Such particles or granules of dye were relatively resistant to the aqueous solutions necessary for nuclear and cytoplasmic staining of the sections. These dyes probably enter macrophages and other cells as an ultramicroscopic dispersion and dye in such a state is probably quite easily "washed out" of sectioned material by water and aqueous solutions of alcohol and stains. It has been observed that suffusions of chlorazol fast pink were removed from macrophages and other cells when

sections were subjected to water, whereas the segregated particles or granules of dye were unaltered by the same treatment (Williams and Hodge, '43). This was true for dye absorbed at the periphery of macrophages as well as for the so-called intracellular suffusions of the vital stain. The term "dypsocytosis" has been applied to the intracellular storage of "excessively minute" particles (Evans and Scott, '21).

If such an interpretation (as above) is used to explain the diminished solubility (in water) of the dye particles deposited within fixed cells which had been injured in vivo it would suggest that some mechanism for segregation, or better, aggregation exists within such cells. It should also be considered that injured cells may contain elements (not present in the normal state) which the dyes truly stain. Evidence to prove or disprove either of these interpretations is not possible from the available data. However, the scattered particles or clumps of dye observed in the injured myocardial fibers were not distributed in a manner that would suggest true staining of intracytoplasmic contents.

It seems correct to assume that whole particles of the size seen in the histological preparations did not enter the fibers. Therefore some type of intrafibral aggregation must have ensued. Whether or not these relatively large particles of dye resulted from the cumulative combination of the dye with cytoplasmic (fibral) constituents or was an expression of a physical or chemical relationship of dye deposited within a fiber to "new" dye entering the fiber could not be determined.

III. Effects of toxic doses of cardiac glycosides upon the myocardium

The histopathology of the myocardial damage induced by massive doses of cardiac glycosides are suggestive of restriction of the arterial blood supply to this tissue and in some instances such lesions have contained true areas of infarction (Bauer, '34; Hu, Lieu and Li, '36; Buchner, '34; Weese and Dieckhoff, '34; Hueper and Ichniowski, '41; LaDue, '42). The coronary vessels did not themselves show changes and

LaDue ('42) has suggested that myocardial ischemia may result from capillary constriction or from increasing the strength and duration of systole thus interfering with coronary flow. Essex and Visscher found that very large doses of digitalis administered to unanesthetized dogs reduced the coronary flow. La Due's work suggests that the effective constriction of the coronary blood supply might be arteriolar because of the small size of the myocardial lesions. There is some indication that these cardiac glycosides may exert direct toxic cellular effects since they apparently produce histological changes in the central nervous system (Weese, '36). Also, several workers have shown that frog muscle loses potassium in ouabain (Cattell, '37; Mangun, Reichle and Myers, '41). However, the fact that the pronounced symptoms and electrocardiographic changes " . . . appear rapidly and are evanescent in character is consistent with the idea that they may be due to arterial spasm" (La Due, '42).

In the present study there was no definite evidence of infarction and the amount of necrosis was very limited. This is not unusual since several workers have observed the more frequent microscopic changes to consist of loss of striations, atypical nuclei, and mononuclear and polynuclear cell infiltration. Large doses of cardiac glycosides have produced in dogs myocardial damage sufficiently severe to eventually result in a considerable amount of fibrosis (La Due, '42). It was also reported that such dogs showed signs of starvation associated with marked anorexia and persistent vomiting and further that the myocardial lesions were suggestive of vitamin B deficiency (La Due, '43). In the present study the animals were well and showed no significant alterations in weight. Therefore it seems safe to assume that a nutritional deficiency was not involved as a cause of the myocardial lesions.

Sufficient evidence is not available at present to demonstrate the extent of the phenomenon of atypical "vital staining" as a dependable and general indicant of cellular damage. Cells which do show abnormal deposition of parenterally administered dyes subsequent to injury would now seem to include

epithelium of the renal proximal convoluted tubules, certain of the nuclei of cell bodies of the central nervous system, some of the leukocytes of the blood, and skeletal and cardiac muscle fibers.

Still unrevealed is the true physical and chemical relationship of the vital dyes to the cytoplasm of the damaged cells. That is, are the dyes staining some cellular element or are they present within injured cells as essentially autonomous entities? The experimental evidence indicated that the entrance of dye into the tissue studied here (myocardial fibers) was a manifestation or result of injury. But, the subsequent intrafibral history of the dye other than its obvious presence there remains obscure.

SUMMARY

Twelve albino rats received daily subcutaneous injections of 0.5 cm^3 of trypan blue or of chlorazol fast pink (0.5% aqueous solution) for 6 days. During this period 7 of these rats were daily injected with 0.25 mg (in 1 cm^3 of 35% alcohol) of digoxin (a glucoside of *Digitalis lanata*). Similarly 9 mice were treated with the same dyes (0.1 cm^3 daily for 5 days) and also received digoxin (0.1 or 0.05 mg subcutaneously daily) during the same 5-day period. In addition 15 mice were treated only with the dyes.

The digoxin caused small diffuse myocardial lesions and the dyes were deposited as small granules or particles within damaged portions of cardiac muscle fibers. Macrophages accumulated at these lesions and stored vital dyes in the usual fashion. Therefore the dyes "indicated" the digoxin-induced myocardial damage in 2 ways: (1) As would be expected, dye-containing macrophages were frequent in the sites of injury. (2) Particles and granules of vital dyes were deposited in the remaining cytoplasm of necrotic portions of heart muscle fibers.

In hearts of non-digoxin-treated animals the vital dyes were limited to macrophages, mesothelial cells, and elastic fibers (slight staining). The findings demonstrated that in-

jured cardiac muscle fibers respond to vital dyes in a manner similar to that observed in damaged epithelial and other cells.

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PLATE 1

EXPLANATION OF FIGURES

All figures × 750. *m* indicates macrophages containing trypan blue, and *d* shows the same dye within damaged myocardial fibers. Figures 1–4 are from sections stained with eosin alone.

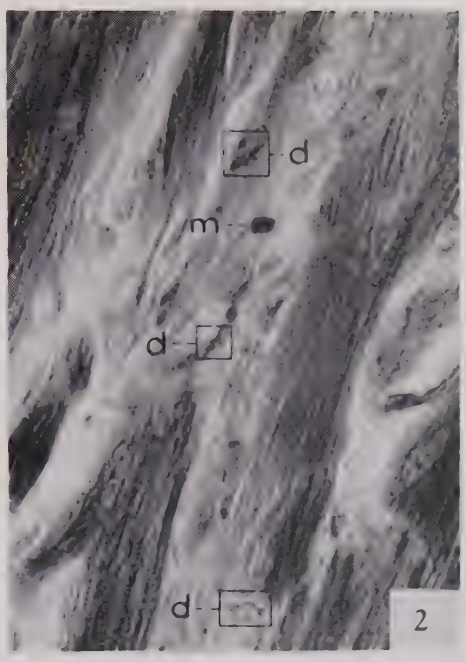
1 Normal myocardium from an untreated rat. There are numerous dye-containing macrophages. No vital dye within muscle fibers.

2 Small area of damage in heart of a digoxin-treated mouse. Very small particles of trypan blue in damaged (very lightly stained with eosin) myocardial fibers.

3 Large amount of dye within a portion (enclosed by rectangle) of a digoxin-damaged myocardial fiber of a rat.

4 Digoxin-injured rat heart. Splitting of myocardial fibers and deposition of dye within damaged portions (site of greatest deposition of dye enclosed by rectangle). Several dye-containing macrophages are in the area.

1



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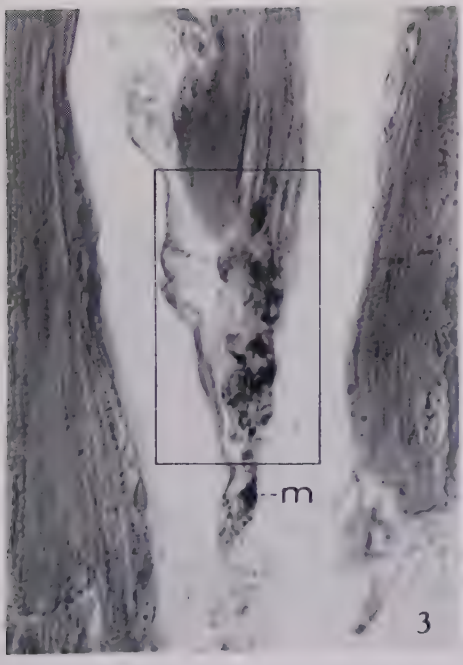

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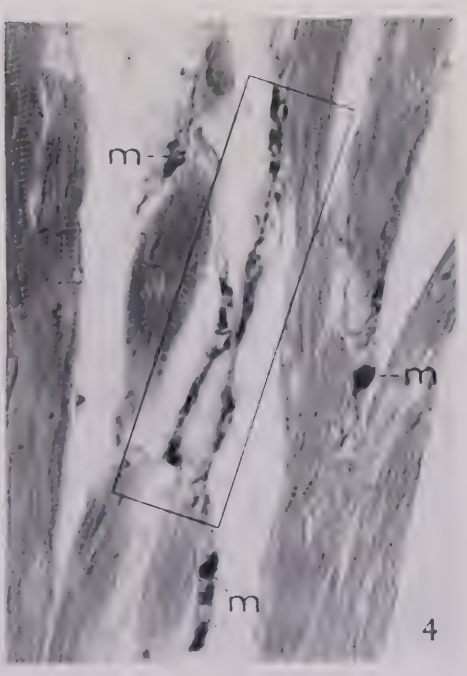

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PLATE 2

EXPLANATION OF FIGURES

All figures × 750. *m* indicates macrophages containing trypan blue and *d* shows the same dye within damaged (by digoxin) myocardial fibers. All figures except no. 8 are from sections of hearts of digoxin-treated rats.

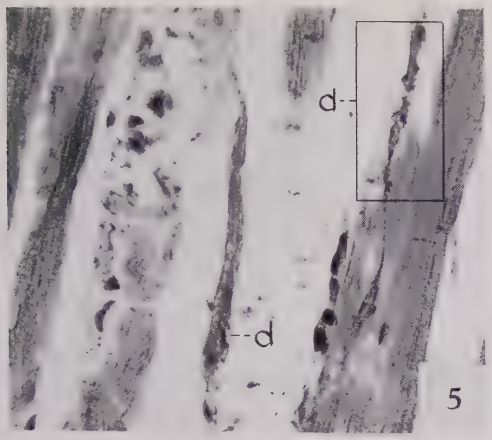
5 Area of myocardium showing dye *(d)* in damaged muscle fibers (Mallory's stain).

6 Small particles of dye within damaged fibers and several macrophages. Eosin-stained section.

7 Numerous dye-containing macrophages. Fibers (myocardial) appear intact and contain no dye. Eosin-stained section.

8 Unstained section of normal rat heart. Note the size of the macrophages and appearance of the segregated dye within them.

9. Unstained section showing a digoxin-produced myocardial lesion. Note the small particles of dye deposited within a damaged fiber.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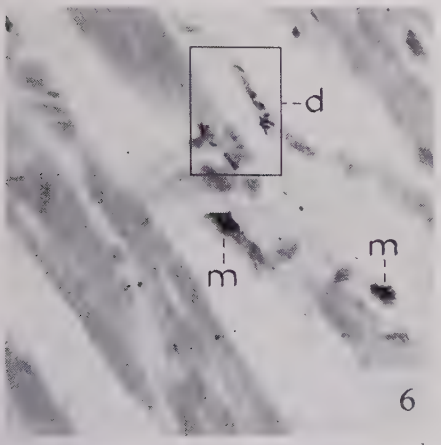
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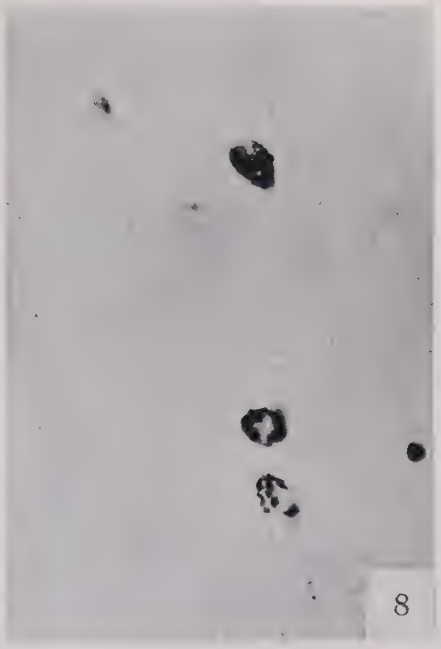
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DESCRIPTION OF BILATERAL CORACO-BRACHIALIS BREVIS MUSCLE, WITH A NOTE ON ITS SIGNIFICANCE

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ONE FIGURE

A number of abnormalities were seen in a subject recently dissected at Aberdeen University. The most interesting feature was the presence of a coracobrachialis brevis muscle on both sides. On the left side, the muscle arose by a fleshy belly from the anterior surface of the coracoid process, at the junction of the horizontal and vertical portions, about 3 cm from the tip of the coracoid. It passed downwards and outwards, with a slight upward concavity, over the anterior surface of the subscapularis. It was covered anteriorly by pectoralis minor, coracobrachialis, and the short head of biceps, and was inserted into the medial bicipital ridge, about 1 cm below the lesser tubercle; a fascial expansion continued into the tendon of insertion of latissimus dorsi. The muscle measured 6.5 cm in length, with a maximum breadth of 1.3 cm. In this instance the insertion of the subcapsularis extended about 3 cm downwards from the lesser tubercle, so that the abnormal muscle was inserted anterior to it. On the right side the muscle arose from the same part of the coracoid process, 2 cm from the tip: it was consequently shorter — 4.3 cm in length, maximum breadth 1.2 cm. Its muscular relations were the same as on the L. side.

Literature relating to this muscle is not abundant. Macalister (1875) deals with it at some length. In both Quain's ('23) and Poirier and Charpy's ('01) textbooks, the muscle is mentioned and reference made to an article by Wood (1867).

This writer had seen the abnormal muscle four times, and quoted references to it by Cruveilhier and MacWhinnie. Wood also described a more common variation of coracobrachialis, which he designated "longus," extending, in its fully developed form, from the coracoid process to the median

Fig. 1 Sketch of anterior aspect of right arm showing anomalous coracobrachialis muscle (*).

supracondylar ridge, or to the median epicondyle. In one subject this latter muscle was seen to "pass entirely superficial to and across the brachial vessels and median nerve, the tendon forming an aponeurotic opening for them . . . as

they cross from the inside to the front of the arm." The resemblance of this arrangement to the lower disposition of adductor magnus is apparent.

In view of the occasional existence of these coracobrachiales longus and brevis muscles, Wood suggested that the coracobrachialis ordinarily described in textbooks of anatomy (he called it coracobrachialis medialis) was the representative of a group of muscles, homologous to the adductor group in the thigh, the short or upper part corresponding to the adductor brevis, the median portion to adductor longus, and the long inferior portion to adductor magnus. In investigations of the comparative anatomy of the group he found great diversity in the incidence of constituents in various animals. In the cat and dog, for example, the brevis muscle was the only representative, as also in bats and moles: the long inferior element was particularly conspicuous in the porcupine and squirrel. A double muscle representing the short and long portions was present in the marmot, beaver and hamster. His general conclusion was that those animals which "use the forearms for distinct prehension, digging, swimming, or climbing, have, as a rule, a larger and more highly developed coracobrachial muscular apparatus."

Variations in the form of coracobrachialis brevis have been recorded. It is given the alternative name of coracocapsularis, on account of its occasional insertion into the capsule of the shoulder-joint. Macalister (1875) mentions that the upper part may arise from the subcapsularis tendon; the lower part may be inserted into the tendon of latissimus dorsi, or into the skin and fascia of the axilla. It may also arise from the coracoid process by a tendon and be inserted into the tendon of subscapularis, or interlace with the fibers of the muscle itself.

I wish to thank Professor A. Low for permission to publish this note and Dr. G. A. G. Mitchell for his helpful criticism and advice.

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MITOTIC ACTIVITY IN THE ANTERIOR HYPOPHYSIS OF OVARIECTOMIZED RATS AFTER INJECTION OF ESTROGENS

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INTRODUCTION

Several investigators (Wolfe, '35; Halpern and D'Amour, '36; Wolfe and Wright, '38) have shown that administration of estrogens will cause an increased mitotic activity in the anterior hypophysis of the rat but it was implied that this occurred only when injections were repeated during a period of weeks or months. In the mouse, Allen, Smith and Gardner ('37) found no increase in mitosis in the hypophysis within 48 hours after a single injection of Theelin. Nevertheless, the fact that there is a marked increase in mitotic activity in the late estrous phase of the sexual cycle of rats (Hunt, '42, '43) indicates that the response of the hypophysis to estrogen may occur within a relatively short time. Consequently, the questions are raised whether 1 or 2 injections of estrogen will increase the mitotic activity and, if so, when the maximum number of mitoses occurs after an injection, how much estrogen is necessary to equal the activity in a normal animal, whether there is a quantitative relationship between the amount injected and the number of mitoses and finally whether the response to estrogen becomes reduced with advancing age.

MATERIAL AND METHODS

The 139 rats used in the experiments were of the Long-Evans strain from the colony of Dr. C. M. Goss. The animals

[1] Aided by a grant from the University of Alabama Research Fund.

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which ranged from 72 to 613 days of age when killed were ovariectomized 20 to 30 days previously. Except for the controls they were injected intraperitoneally with either Theelin, Progynon-DH or Progynon-B.[2]

After killing the animal by decapitation, the brain was removed and the hypophysis fixed while still attached to the base of the skull in Allen's B-15 fluid. Paraffin sections were cut at 3 micra in the coronal plane of the gland and stained with Masson's hematoxylin, acid fuchsin, ponceau de xylidene and aniline blue.

The mitotic activity was determined by counting all mitoses in a section of known area. The area was calculated after projecting and tracing the outline of the section at a magnification of 50 diameters and determining this area by means of a planimeter. The position of the dividing cells in the section was plotted on this tracing in order to see if the distribution of dividing cells was essentially equal. The average area of all sections examined was about 5 mm^2 but since the area of a section varied considerably due to variation in size of the gland or level examined, mitotic activity was expressed in the number present per square millimeter of section rather than the number per section. There were approximately 10,000 cells per square millimeter and since the distribution of dividing cells was quite uniform in different parts of the anterior lobe, it was considered that the area examined, which included 40 to 60,000 cells, was adequate for determining the mitotic activity.

RESULTS

Time after injection of increased mitotic activity

Increased mitotic activity may occur in the hypophysis from 41 to 98 hours after an estrogen has been injected. Animals killed within 24 hours of a single injection (group 2, table 1) show no increase in mitotic activity over the controls (group 1,

[2] The Theelin and Progynon were generously supplied through the courtesy of Parke, Davis and Company and the Schering Corporation respectively. Theelin (estrone) is abbreviated to T in the tables, Progynon-DH (α-estradiol) to PDH and Progynon-B (the benzoate ester of α-estradiol) to PB.

table 1) and of those receiving 2 injections 41 and 19 hours before death (group 1, table 2) only 1 of 4 animals shows more than a slight increase. It has 6.7 mitoses per square millimeter and had it been comparable to the others the average would have been essentially like that of the control group. This shows, however, that occasionally mitotic activity occurs as early as 41 hours after an injection.

If the animals are not too old and a sufficient amount of estrogen is injected, most animals have an increased mitotic

TABLE 1

Mitotic activity in the hypophysis after 1 injection of estrogen.

GROUP	NUMBER OF ANIMALS	AGE, AVERAGE AND RANGE	INJECTION	QUANTITY INJECTED	TIME OF INJECTION, HRS. BEFORE DEATH	MITOSES PER MM^2 MEAN ± S. E.
		days		*μg*		
1	5	117 (106–121)				1.2 ± 0.25
2	6	105 (102–109)	T,PDH	20–300	24	1.5 ± 0.40
3	2	112 (72–151)	PDH	2	48	2.5 ± 1.20
4	3	90 (80–108)	T,PDH	20	48	6.9 ± 2.31
5	5	119 (80–163)	T,PDH	200–300	48	13.9 ± 3.20
6	5	426 (382–520)	T,PDH	20–400	48	1.0 ± 0.07
7	4	162 (132–237)	T	15	72	1.8 ± .47
8	4	125 (81–142)	T	40–50	72	28.1 ± 28.80
9	12	118 (81–149)	T,PDH	150–240	72	16.5 ± 4.07
10	5	161 (144–177)	T,PDH	300–1200	72	17.6 ± 2.00
11	13	401 (196–613)	T,PDH	100–400	72	1.9 ± 0.54
12	3	142 (82–174)	T,PDH	200–600	98	12.6 ± 7.04
13	2	173 (171–174)	T	300–600	120	0.9 ± 0.07

activity within 48 hours (groups 4, 5, table 1), although it may be just beginning to increase since in some cases there is a preponderance of early division figures. This is later than was anticipated since Allen, Smith and Gardner ('37) found in the mouse that after a single injection of Theelin, mitotic activity is increased in the genital tissues prior to 9½ hours and reaches a maximum within 37 hours. It seems probable that their failure to find increased mitotic activity in the hypophysis was due to the fact that only 2 animals were al-

lowed to live as long as 48 hours after an injection and none for a longer period.

Most animals killed 72 hours after 1 injection also show increased mitotic activity (groups 7, 8, 9, 10, table 1), but there is a great variability in response. One animal 81 days of age that received 40 μg of Theelin has 102 mitoses per square millimeter. Another of 89 days that received 200 μg has only 3.5 per square millimeter. It is believed that some of the animals with low counts might have shown a higher mitotic activity if they had been killed sooner.

Mitotic activity is likewise increased in 2 of 3 animals that received a single injection 98 hours before death (group 12, table 1). The one that did not show an increase was younger (81 days) which may indicate that the older animals do not inactivate or eliminate the estrogen as rapidly as the younger ones.

Neither of the 2 animals (group 13, table 1) that received 1 injection 120 hours before death shows an increased mitotic activity.

Thus mitotic activity was found to be increased in some but not all animals as soon as 41 hours and as late as 98 hours after a single injection. Some animals apparently have a shorter or longer than average period of increased activity or the time that increased activity occurs after an injection may vary. In order to avoid killing the animals when mitosis had not started or had already stopped, 2 injections were made in a number of experiments (table 2) at the times found most effective with single injections, i.e. 72 and 48 hours before death.

The appearance of the vaginal smear proved to be a valuable indication of whether increased mitotic activity might be found in the hypophysis. In animals that show marked activity the smear is of the late estrous or early metestrous type. This is true of both the injected animals and the normal ones which have typical estrous cycles (Hunt, '42).

Quantitative relationship between amount of estrogen injected and mitotic activity

In groups of animals of comparable age the average number of mitotic figures in the hypophysis increases up to a certain point as the amount of estrogen injected is increased. Animals receiving single injections show this to a certain extent (groups 3, 4, 5, and 7, 8, 9, table 1) but since the results are more uniform in the groups receiving injections 72 and 48 hours before death (2, 3, 5, table 2), these will be considered in more detail.

TABLE 2

Mitotic activity in the hypophysis after 2 or more injections of estrogen.

GROUP	NUMBER OF ANIMALS	AGE, AVERAGE AND RANGE	INJECTION	QUANTITY INJECTED	TIME OF INJECTION, HRS. BEFORE DEATH	MITOSES PER MM^2 MEAN ± S. E.
		days		*μg*		
1	4	113 (112–114)	T	2 · 25	41, 19	3.3 ± 1.32
2	4	108 (104–111)	T	2 · 2.5	72, 48	1.2 ± 0.29
3	9	95 (74–109)	T	2 · 25	72, 48	7.3 ± 1.51
4	9	291 (295–316)	T	2 · 25	72, 48	1.7 ± 0.49
5	4	81 (74–83)	T	2 · 250	72, 48	30.3 ± 3.53
6	4	281 (214–320)	T	2 · 250	72, 48	3.7 ± 1.85
7	10	105 (92–116)	T	7 · 12, 13	78–6	16.6 ± 3.27
8	4	89 (86–95)	PDH	2 · 25	72, 48	5.6 ± 2.31
9	10	83 (76–90)	PB	2 · 25	72, 48	22.1 ± 5.46
10	6	79 (78–81)	PB	2 · 250	72, 48	30.9 ± 4.52

Animals with injections of 2.5 μg of Theelin have an average of 1.2 mitoses per square millimeter which is not significantly different from the number found in ovariectomized controls (group 1, table 1). All animals of the group showed an estrous response judged by the vaginal smear although only 1 had a cornified smear at the time of death.

When the amount of Theelin injected is increased to 25 μg mitotic activity increases to an average of 7.3 mitoses per square millimeter. In the group of 9 animals the number ranges from 2 to 16. The animal with the lowest count and 1 other with a count of 4.9 did not have a cornified cell smear when killed and if these are excluded the average count would

be 8.4. This falls short of the number that would have been obtained had mitoses increased proportionally to the increase of estrogen.

Injections of 250 μg of Theelin increases mitotic activity to an average of 30.3 per square millimeter with a range from 22 to 35. Although individual animals may have more than 30 mitoses per square millimeter, this average figure is apparently the maximum for this age group since it has not been exceeded after larger injections or with variation of the type of estrogen used. Moreover, it corresponds closely to the average mitotic activity occurring in the hypophysis of normal animals of the same age killed in the late estrous phase of the sexual cycle (Hunt, '43).

Response to estrogen by animals of different ages

There is a much greater variation in response to estrogen by animals of different age groups than there is individual variation in a group of the same age. The response of 18 older animals (group 6 and 11, table 1) to single injections of estrogen ranging from 20 to 400 μg (16 of 18 had 100 μg or more) is uniformly low. The difference in response of young and old age groups is even more clearly shown by comparing results from animals receiving 2 injections (group 3 with 4, and 5 with 6, table 2). In the older (10 months) animals receiving 25 μg of Theelin the average mitotic activity is 1.7 and in those receiving 250 μg it is 3.7. The younger (3 months) animals have 4 and 8 times as many mitotic figures, respectively, as the older ones receiving the same amounts of Theelin. Statistically the probability that the difference in the response of the 3- and 10-month-old animals is due to chance is less than .01.[3]

[3] The probability (P) is determined by the formula given by Fisher for the significant difference of means of small samples. In the present work I consider that a P of more than .05 indicates that a difference is not significant, between .05 and .02 that it is doubtfully significant, between .02 and .01 possibly significant, and .01 or less almost certainly significant. A P of .01 means that once in a 100 times the difference may be due to chance.

Reaction to different estrogens

In comparing the effect of the 3 estrogens used in the experiments the average mitoses per square millimeter after 2 25 μg injections are as follows: Theelin, 7.3; Progynon-DH, 5.6; Progynon-B, 22.1 (groups 3, 8, 9, table 2). There is no significant difference (P = 0.5) in the results after injections of Theelin and Progynon-DH but between Theelin and Progynon-B the difference is possibly significant (P = less than .02). Progynon-B, which is the benzoate ester of estradiol, is considered to have a more prolonged action and this may explain the higher mitotic activity attained with it. The group having Progynon-B shows great variability, the mitoses per square millimeter ranging from 53.4 to 5.5. Of the 10 animals in the group 3 are well above average and 4 well below. It is this wide variation that reduces the possibility of a statistically significant difference between Theelin and Progynon-B.

With 2 injections of 250 μg of Theelin or Progynon-B the average mitotic activity of the 2 groups (5 and 10, table 2) is 30.3 and 30.9 respectively. This close correspondence of results is not necessarily an indication of similarity of potency of the 2 estrogens. Since an average of about 30 mitoses per square millimeter is apparently the maximum attainable, the amounts injected may be in excess. As noted above, injections of 25 μg of Progynon-B result in a mitotic activity of 22.1 which is almost the maximum. In fact there is no significant difference statistically (P = .25) between group 9 having 25 μg injections of Progynon-B and group 10 having 250 μg. On the other hand the difference between groups 3 and 5 having 25 and 250 μg of Theelin respectively is definitely significant (P = less than .01). From this it is concluded that 250 μg of Progynon-B is in greater excess than is 250 μg of Theelin in producing maximum mitotic activity and consequetnly the 2 estrogens are not the same in potency.

Effect of estrogens given from 78 to 6 hours before death

Since there is a considerable lag between the time of injection and the increase in mitotic activity, it seemed possible that the activity might follow a decline in the level of estrogen. To test this possibility 12 or 13 μg of Theelin were injected on the following hours before death: 78, 70, 54, 46, 30, 22, 6 (group 7, table 2). Judging from the other experiments the estrogen received during the last 30 hours would not be effective so that the first 4 injections which totaled 50 μg and which were given 78 to 46 hours before death, would be comparable to that given to group 3 (table 2). The average mitotic activity of group 7 (table 2) is 16.6 mitoses per square millimeter as compared with 7.3 for group 3. Thus mitotic activity is obviously not due to a decline or elimination of the estrogen. The higher average may be due to the more frequent injections which would maintain a more constant effective concentration and give less chance of killing the animal at a time when mitotic activity was rising or declining.

DISCUSSION

Before an injected estrogen is effective in increasing the mitotic activity of the hypophysis of ovariectomized rats, it is necessary that it have a sufficient level of concentration and be present 40 to 50 hours. A low concentration, just sufficient to cause an estrous response, is not adequate to show definitely an increase in mitotic activity. In order to produce a response in mitotic activity equivalent to the maxmium in the hypophysis of a normal animal during the estrous cycle, it is necessary to inject 250 to 300 Rat Units (250 μg of Theelin or 25 μg of Progynon-B) of estrogenic substance. In animals 3 months of age this maximum activity is 30 mitoses per square millimeter and larger amounts of estrogen become ineffective in causing a further increase in mitotic activity.

The effective concentration of the estrogen 40 or 50 hours after injection presumably varies due to individual differences in the rate of elimination or inactivation and this undoubtedly is one of the factors that accounts for the variability in the mitotic response where factors such as amount of estrogen, time after injection and age of the animal are kept constant.

Another important factor in the variability of response is the varying degree of susceptibility to division of cells of the hypophysis. This is apparently the case not only in the hypophysis of different animals because of hereditary and age differences but also in the same hypophysis. Thus the majority of divisions occur in the chromophobes although division is not entirely limited to this type of cell. Furthermore, the fact that more cells divide with injections of 250 μg of Theelin than with 25 μg indicates that some must be more susceptible than others to division. This susceptibility is not due to an obviously more favorable location of the dividing cells since they are uniformly distributed in the lobe and are in no special relation to blood vessels.

Another indication of varying susceptibility is the occurrence in a few animals of unusually high or low mitotic activity. In 5 to 10% of the animals 3 or 4 times as many mitoses occur as are expected judging from the usual response. Likewise unusually low counts are encountered. This is true in both normal animals (Hunt, '43) and in the present series of injected ones. In a series of long term interrupted injections of estrogen Wolfe and Wright ('38) found 2 of 8 animals that showed adenomatous changes in the hypophysis. The others were apparently less susceptible to the estrogen.

The cells of the hypophysis become increasingly less susceptible to division as the animal advances in age. In the normal series (Hunt, '43) it was assumed that the cells of the hypophysis became more refractory to division with advancing age since there was no evidence that the ovaries produced less estrogen in the older animals. This idea is supported by the present experiments since the same amount of estrogen

results in 4 to 8 times as many mitoses in an animal of 3 months as in one 10 months of age.

The mitotic activity resulting from injections of different estrogens indicates that Progynon-B is more effective than either Theelin or Progynon-DH. This may be due to the more prolonged activity of Progynon-B rather than an intrinsic difference in its action. The average mitotic activity resulting from 7 injections of 13 μg of Theelin at 8 to 16 hour intervals is essentially the same as the activity after 2 injections of Progynon-B at 24 hour intervals. The 2 groups are considered comparable even though a greater total amount of Theelin was given inasmuch as the effective period of action is considered to be between 48 and 72 hours before death and during this period the 2 groups received essentially the same amount of estrogen.

The mitotic activity after injections of Theelin between 6 and 78 hours prior to death also indicates that the mitosis is not due to withdrawal of the estrogenic substance.

Elsewhere in the body cellular injury seems to be a factor that results in increased mitotic activity, and in the hypophysis there is a possibility that division follows the death of cells due to over-stimulation by estrogen. However, it is difficult to see evidence of such cellular injury and, if it does occur, replacement must rapidly exceed the loss since a considerable hypertrophy of the gland occurs with repeated injections of estrogen (Wolfe and Wright, '38).

At present it seems most likely that the estrogen affects the cells directly by modifying the physicochemical activity of the cell in such a way that the process of division is started.

In whatever way the estrogen does act to cause an increase in number of cell divisions, its activity as far as division is concerned is confined to the reproductive system and hypophysis and possibly indirectly through the hypophysis to the suprarenal and thyroid glands. There is no evidence that it affects the cells of the digestive or integumentary systems or other systems where division commonly occurs.

SUMMARY AND CONCLUSIONS

1. Mitotic activity may be increased in the anterior hypophysis of ovariectomized rats between 41 and 98 hours after injections of estrogens.

2. In order to equal the number of mitoses found in the hypophysis of normal rats in the post-estrous phase of the sexual cycle, it is necessary to inject 250 to 300 Rat Units of Theelin or Progynon-B 72 and 48 hours before death.

3. In animals 3 months of age such injections result in an average appearance of 30 mitoses per square millimeter of sections 3 micra in thickness. Larger amounts do not cause a further increase. With smaller injections the number of mitoses is proportionally smaller. Injections of 2.5 Rat Units, which is sufficient to cause an estrous response, does not cause an appreciable increase in mitotic activity.

4. As the animals become older, the pituitary cells become increasingly refractory to the mitotic stimulating effect of estrogens. There are 4 to 8 times as many mitoses in 3 month animals as in those of 10 months receiving an equivalent amount of hormone.

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MECHANICS OF INVAGINATION

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NINETEEN FIGURES

INTRODUCTION

All cells have superficial gel layers that exert continuous contractile tension, a fundamental property of protoplasm when in the gel state. Gel layer and endoplasm are different states of the cytoplasm that readily change from one state to the other. Local changes of the contractile tension of the gel layer of free isolated cells result in changes of cell form and in locomotion. When cells are firmly adherent to one another in sheets and act together quite different phenomena, invagination for example, result from local changes of contractile tension of the gel layers of adherent groups of cells.

When considering roles that the superficial gel layer of cells might play in the development of Amblystoma it seemed probable that the concave surfaces of such invaginations as blastopore, neural tube, lens, otic vesicle, nasal pit, optic vesicle and optic cup might in some manner be produced by an increased contractile tension of the superficial gel layers of the cell surfaces bordering the concavities. The ends of the cells bordering the cavities are smaller than the opposite ones. After some speculation I consulted Dr. Park Miller, Department of Physics, University of Pennsylvania.

According to Dr. Miller an increase of the tension on one side or a decrease on the opposite side of a series of adherent segments that resist distortion will result in a concave depression on the side of the greater tension. Such a system

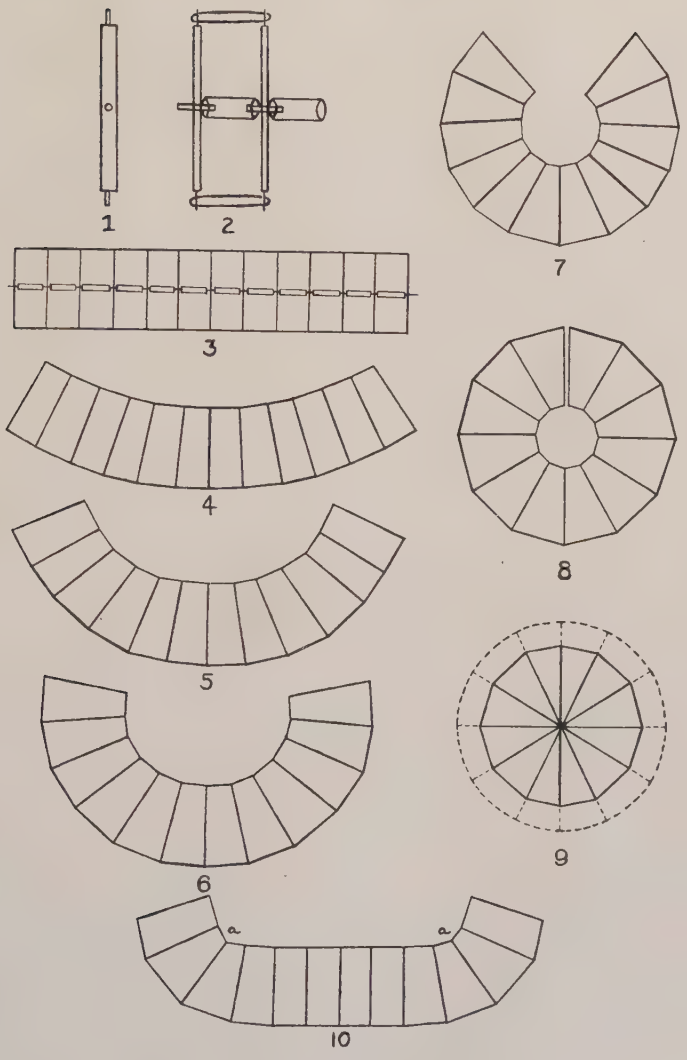
attains a state of equilibrium or stability with the least distortion of its component elements and least display of energy. There is less distortion of each segment when the contracting surface becomes concave and the opposite surface convex. The concave-convex form is the resultant of the 2 forces, an increase or a relative increase of contractile tension on 1 surface and the resistance of the segments to distortion.

Invagination model

Dr. Miller kindly designed and had a model made for me in the physics shop. It consists of 13 brass bars, $5 \times \frac{1}{2} \times 1/16$ inches (figs. 1, 2), with a peg at each end and a short one on each side at the center. Two inch pieces of stiff 7/16 inch rubber tubes fit loosely over the center pegs to keep the bars apart. The center pegs serve to keep the pieces of tube in position. A series of rubber bands stretched from one end peg to the next one holds the bars together in a row (fig. 3). The model should be laid on a smooth table to reduce friction. Strips of wood with nails for pegs make good models.

Such a model represents a cross section of a series of cells firmly adherent at their contact surfaces, the bars. The rubber bands represent the gel layers at the ends only. The pieces of tube represent incompressible, less viscous endoplasm.

When the tensions of the rubber bands are equal along the 2 sides, the model is straight (fig. 3). By increasing or decreasing the tension uniformly (increasing or decreasing the number of rubber bands per segment) along one side, the model becomes concave on the side of the greater tension and comes to rest in equilibrium (figs. 4–7). The shape of the concave curve (invagination) depends upon the difference in tension on the 2 sides. With sufficient tension a complete circle results (fig. 8). It is easy to see how with an ideal model still more contraction would reduce the ends to points, eliminate the central cavity and elongate the segments (fig. 9). If instead of a uniform increase of tension along 1 side of the model, local increases are produced different shaped concavities result (fig. 10).



Figures 1 to 10 from invagination model

Fig. 1 Flat bar with a peg at each end and on each side.

Fig. 2 Two bars in position. A rubber band at each end, a rubber tube spacer in the middle.

Fig. 3 Model with the tension of the rubber bands equal on the 2 sides.

Figs. 4–8 Equilibrium curves with different numbers of bands on the 2 sides.

Fig. 9 Idealized condition with lumen obliterated by still greater increase of tension. Dotted line for no decrease in size of segments.

Fig. 10 Local increase of tension at a and a, neural fold imitation.

Cellular invaginations arise from limited areas of epithelial membranes that exert contractile tension in all tangential directions and hence exert pulls on the edge of the invagination plate that oppose invagination. Epithelial membranes are more or less attached to neighboring tissues and this also opposes invagination. In order to imitate the living the end bars of the model should be held in position. It might be better to use a much longer model for then friction might imitate the attachments of the membrane to neighboring structures.

If the end bars are held in position, invagination does not occur no matter how many more bands there are on 1 side than on the other, provided each segment of a side has the same number of bands. Thus if each segment has 3 bands on the upper side and 1 on the lower side the model will be straight as in figure 3. One may put 4 or 5 or even 6 bands on each segment of 1 side without changing the position 3, provided the end bars are held in position. If end bars are released the model immediately curls and assumes a position as in figure 6. If the end bars are now pulled outwards, positions as in figures 5 and 4 are attained as well as intermediate ones. With proper outward pull position 3 can again be attained.

If the end bars are held in position 3 with 1 band on each segment and 2 bands are added to each upper end of the 4 middle segments the upper side becomes slightly depressed; the 4 upper bands of the middle segments shorten and the 4 lower ones lengthen. The 4 segments at each end represent non-invaginating epithelium; their upper bands lengthen and lower ones shorten. If the end bars are now released the depression increases and the 4 outer segments at each end are swung towards the concave side and all their bands become equal in length.

If the end bars are held in position figure 3 with 3 bands on each upper and each lower segment and 2 bands are removed from the lower ends of the 4 middle segments the same results are attained as in the preceding experiment.

If fewer segments are involved the depression is reduced. If more segments are involved the depression will increase provided a few outer segments are not changed.

The model differs from cells in that the bars act as levers with the cross tubes as fulcrums. If the upper end of a segment shortens the lower end must lengthen because of the lever action. If the lower end of a segment is weakened it will be lengthened because the now greater tension at the upper end will pull the upper ends of the bars together and spread the lower ends, the cross tubes acting as fulcrums for the levers.

In cellular invaginations the weaker end expands or lengthens because endoplasm is forced in that direction; there is no lever action.

Model simplified

The principle involved can be illustrated with 2 segments of the model (fig. 12). In position (1) the 2 rectangular segments are at rest with 1 rubber band at each end of each segment exerting equal tensions. Position (2) was assumed when the 2 upper bands were doubled. Each upper end is shortened, the middle bar is depressed (invaginated), the lower ends are lengthened. The cross tubes act as fulcrums for rotation of the outer bars. The tension of the upper bands balances that of the lower ones. To distort the system as by pulling or pushing the upper or lower ends of the outer bars in or out or by pushing the middle bar up or down requires force and the moment the force is released the system returns to position (2). Position (2) is that of minimum potential energy. Work is necessary to alter it.

Cellular invaginations

The essential factors for invagination are an increase or a decrease of the contractile tension of the superficial gel layers on 1 side of a group of adherent epithelial cells that offer some resistance to distortion. It should be borne in

mind that gel layers exert continuous contractile tension and that a local increase or relative increase (if there is a decrease on the opposite side) of its thickness and/or viscosity will be followed by contraction of the area until an equilibrium is established.

Most invaginations appear to be due to an increase of the contractile tension of the gel layers on the concave rather

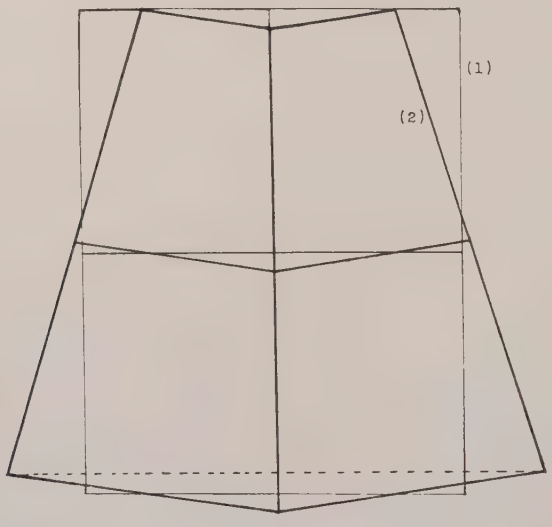


Fig. 11 Two segments of model. Position (1) the 4 rubber bands exert equal tension. Position (2) rubber bands on upper ends doubled. The segments come to rest with the middle bar depressed or invaginated. Work is required to distort the system.

than to a decrease on the convex surface. Both or either surface may be involved but invaginations will be explained as if due to increase on the concave side.

Antagonistic pulls. Invaginations arise from limited areas of epithelial membranes that are in a state of tension. The surface gel layer of each cell exerts contractile tension in all tangential directions and since they are adherent to one another the whole membrane exerts contractile tension in all tangential directions. Pulls will thus be exerted on the edge of the invagination plate that will oppose bending inward

of its edge. Invagination must overcome this tangential pull on its periphery. If an invagination plate forms a vesicle neighboring epithelium will be pulled until it closes over it. Antagonistic pulls continue throughout invagination and if at any time the 2 pulls balance invagination will cease. Epithelial membranes are more or less adherent to neighboring structures. These adhesions oppose invagination in that they oppose the pull of the contracting edge of the invagination plate on the neighboring adherent epithelium.

The relation between the strength of the 2 pulls has something to do with the time involved for an invagination and the shape of the organ. For example, the edges of the neural plate are continuous with the general ectoderm. If the latter is cut along a line parallel to the neural folds on 1 or both sides the wound gaps open and the neural folds move more rapidly than normal towards the mid-line and meet long before the normal time. This indicates that the general ectoderm exerts contractile tension in all directions and opposes the invagination of the neural plate. During invagination the surrounding ectoderm in spite of the opposing tension is pulled to cover the surface area originally occupied by the neural plate. It seems probable that other invaginations are similarly opposed and modified by such pulls.

There are many unexplained problems connected with this aspect of invagination. What happens to the cells of the surrounding membrane as they are pulled? Are they stretched and flattened? What happens to their gel layers? To what extent are more and more remote cells involved? Would antagonistic pulls of neighboring epithelium prevent invagination if there were no increase on the concave but a decrease on the convex surface of the contractile tension of the gel layers of the plate? Would the superficial end of a single cell in a membrane be able to contract to smaller dimensions if its contractile tension and that of its neighbors remained unchanged as the contractile tension of its deep end decreased?

Invagination plays an important role in the development of a variety of organs that differ from one another in shape,

size, structure, cellular composition, location, origin and function. Many factors are involved in each invagination. Invagination areas or plates differ in size, shape, thickness, location and environment. Changes occur in them and in their environment during invagination. Their cells differ in number, size, shape, adhesiveness, viscosity, mitotic rate, growth rate, cytoplasmic differentiation, etc. The above factors are involved in every invagination and play a part in modifying and determining its character.

Invaginations continue until an equilibrium is established either without closure as in organs with ducts or when the edges of the plate meet and adhere to form a vesicle or when the inner ends are reduced to points and the vesicle becomes solid.

Decrease of the concave surface area depends upon the amount of contraction exhibited by the gel layers on the cell ends facing the surface and upon migration, if it occurs, of cells from the surface.

The expansion of the deep or convex surface of invaginating plates is due to the squeezing of endoplasm and nucleus towards the deep part of each cell by contraction at the concave surface end and in addition to the migration of cells from the concave towards the convex surface in some invaginations. As the deep end of each cell expands the peripheral edge of the plate is pushed or swung around towards the closure aperture.

The contractile strengths exerted by the gel layers of different types of cells probably differ and may vary during invagination as differentiation proceeds and changes the chemical composition of the cytoplasm of the cells involved and the composition of the environment.

The resistance to distortion of an individual cell during invagination depends upon the strength of the contractile tension of that part of the gel layer facing the concave surface in relation to the strength of the contraction over the rest of the cell, upon the viscosity of the endoplasm and upon the firmness and extent of adhesion to neighboring cells.

The timing of contraction of individual cell gel layers comprising an invaginating surface may be approximately uniform and simultaneous or otherwise. Uniform simultaneous contractions would give spherical curves. A lack of simultaneous onset and uniformity of timing and strength of the contraction of the gel layers of the concave surface would result in various invagination shapes that change during the process.

Flask cells and migration. The formation of flask cells with long necks is due to excessive contraction of the gel layer at the invaginating end of a cell. The migration of many of them away from the concave towards the convex surface is common and modifies the shape of the invagination curve and reduces the concave surface area. As cells migrate away from the surface neighboring cells are pulled to the very last point of departure and there is never any gap or hole at the surface. The migration of cells out of the plate into neighboring tissues probably does not occur except in the blastopore region and possibly in the presumptive mesodermal invagination grooves of some amphibia.

The migration of single cells from the concave towards the convex surface is clearly evident in sections of some invaginations. The concave surface end of such a cell probably exerts more contractile tension than its neighbors as it decreases in area and contracts against the pull of neighboring cells. This would seem to indicate that other cell-ends facing the same concave surface exhibit the increased contraction responsible for the invagination.

Examples of invagination

The following examples from Amblystoma maculatum indicate that the invaginations are due to increased contraction of the gel layers of the cell ends facing the concave surface. Each invagination has its own peculiar characteristics that involve many modifying factors. The photographs show that the ends of the cells abutting the concave surfaces are contracted as compared with those at the larger convex surfaces

and that the cells extend radially from the concave surface. I am indebted to Dr. Jean Piatt for the loan of his sections from which the photographs were made.

Neural plate invagination or folding

The following quotations from Gillette ('44) which are based on an extensive study "of changes in ectodermal volume, cell number, and cell size during neurulation in Amblystoma maculatum," indicate that my concept of neural plate folding is in accord with his.

P. 203. "The data indicate that the thickening is accomplished by a tangential contraction of the plate towards the dorsal mid-line with a corresponding expansion and thinning of the non-neural epidermis. The folding of the plate appears to be a continuation of this process which proceeds at a greater rate at the external surface than in the depths of the plate." P. 223. "It is proposed that the folding of the plate is a direct result of the one-sided nature of the contraction process which proceeds at a greater rate at the external surface than in the depths of the plate. This suggests that the surface coat of Holtfreter is an important factor in neurulation as well as in gastrulation."

The contraction process of Gillette is due to an increase of the contractile tension of the adherent superficial gel layers on the dorsal surfaces of the elongated and mutually adherent cells of the neural plate. The surface coat is superficial gel layer with a special modified surface.

Dissections of neural plates show that its cells are firmly adherent to one another throughout the thickness of the plate. Adhesion seems to be especially strong at the dorsal surface where the deeply pigmented, thickened gel layers abut one another.

The conversion of the thin convex presumptive neural ectoderm into a thick walled tube is an extremely complex process. A tangential contraction of the area (plate) towards the dorsal mid-line is, according to Gillette, probably responsible for the thickening of the plate. Other factors also play a role. The

pull of the chordaderm on the neural area during gastrulation tends to elongate the plate and to concentrate it towards the mid-dorsal line. Owing to its thickness, relatively great size, and peculiar shape its invagination is much more complicated than that of the lens which comes from simple one-celled epithelium.

Examination of cross sections, figures 12 to 15 of neural plates of Amblystoma of different stages, shows that the ends of the cells facing the concave or dorsal surface become more and more deeply pigmented as the ends contract and become smaller and smaller. Contraction and inward elongation concentrates the pigment of the gel layer. The entire concave surface becomes smaller and smaller in area as the curvature increases and the neural folds are bent towards the mid-dorsal line. At the time of closure the canal wall of the neural tube is relatively small as compared with the area of the wide open medullary plate. In some regions the central canal is almost eliminated by the extreme contraction of the gel layer ends and the migration of many cells from this surface, figure 15.

The dark pigmented streaks extending from the concave surface into the depths are the long pigmented necks of flask cells that have moved varying distances from the surface but still retain their connection with it by slender pigmented strands. They become more and more numerous as the concavity becomes more and more marked. The pigmented necks lose their connection with the surface and disappear after closure. The conversion of surface cells by elongation into flask cells indicates extreme contraction of their superficial ends. As these ends contract and finally leave the surface the adherent ends of neighboring cells are pulled together to fill in the potential gap.

This inward elongation of surface cells into flask cells is one of the earliest indications of an increase of the contractile tension of their gel layers. It becomes marked, when the neural plate is still almost flat, along a deeply pigmented line, (fig. 12) often slightly grooved, that develops in the plate near to and parallel with its outer edge. The contraction of the

gel layers along this line causes a bend, neural fold bend. Dissections along this line of both fresh and formalin material reveal many flask cells with long necks and the firm adhesion of cell to cell.

It is evident from figures 12 to 15 and from other sections of different levels at various stages that there is a lack of uniformity in timing, strength and location of the contractions of the superficial ends of cells in different regions at different stages. This lack of simultaneous behavior is responsible in part for the many different contours along the length of the organ during neurulation.

As the dorsal surface of the plate becomes more and more concave and reduced in area, the deep surface becomes more clearly defined, more and more convex and increased in area. This is brought about by forcing endoplasm towards the convex surface by contraction of the surface gel layers and by the migration of cells from the concave towards the convex surface.

The above phenomena indicate that the gel layers of the adherent cells on the concave surface exhibit increased and continuous contractile tension and that this is responsible for the invagination of the neural plate into the neural tube.

Fig. 12 Neural plate, slightly concave, slightly bent where dark necks of several flask cells extend inward, stage 16, about 80 dia.

Fig. 13 Neural plate, anterior to figure 12. Invagination curve with deeply pigmented cell ends. Dark necks of flask cells extend deep into plate.

Fig. 14 Posterior region neural groove, concave surface greatly reduced by contraction of gel layers and inward migration of many cells with long pigmented necks, stage 20.

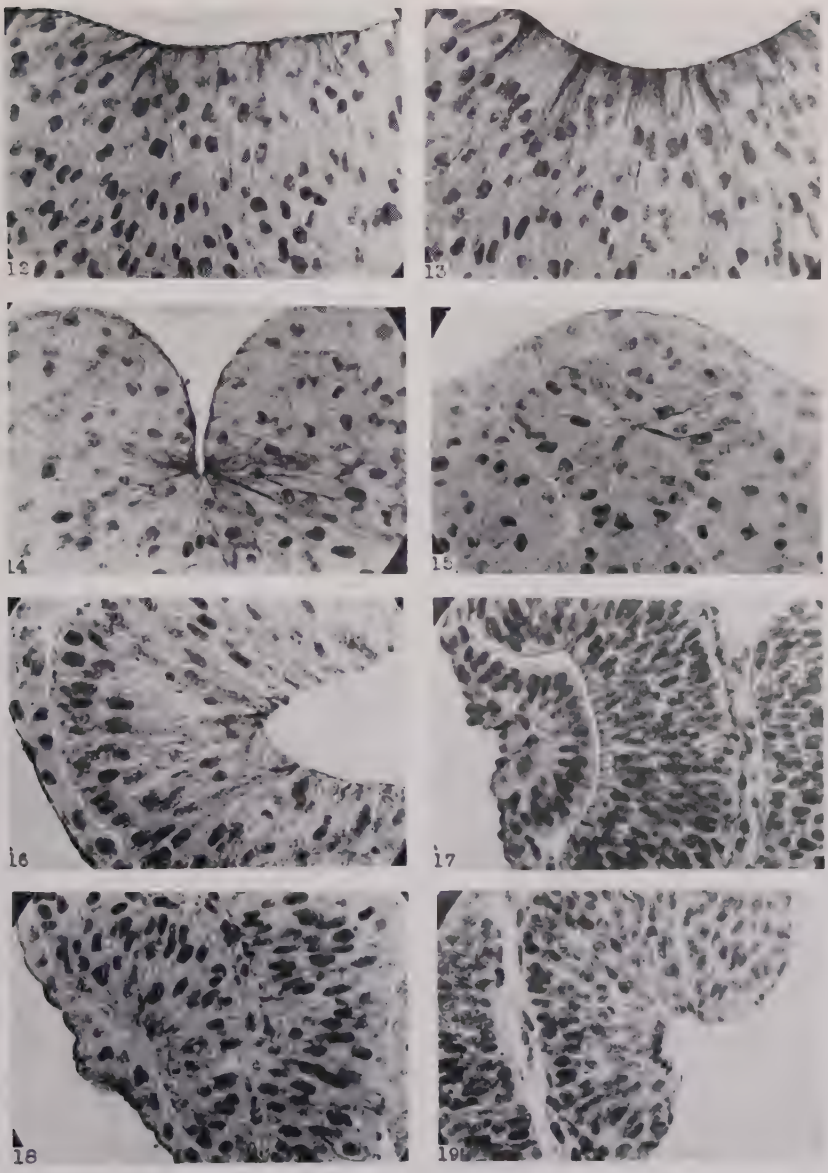
Fig. 15 Neural tube posterior region. Lumen almost obliterated by contraction of concave surface and migration of cells away from it. Pigment about gone. Stage 21.

Fig. 16 Optic vesicle evagination. Ends of cells at concave smaller than those at convex surface. Pigment about gone. Stage 21.

Fig. 17 Lens invagination, concave surface smaller than convex. Outer layer ectoderm crumpled by invagination of inner layer. Optic cup invagination. Stage 35.

Fig. 18 Ear invagination. Small concave and larger convex surfaces with radial wedge-shaped cells. Outer layer of ectoderm crumpled. Stage 25.

Fig. 19 Nasal pit. Note small pigmented necks of flask cells bordering the concavity. Stage 36.



Figs. 12 to 19

Neural tube bendings

The arrangement of cells in the early neural tube, approximately perpendicular to inner and outer surfaces, makes it an ideal organ for bendings. The cells are firmly adherent to one another. Cross sections through bends show cells with small ends at the concave and larger ends at the convex surface. This indicates that there is an increase of the contractile tension of the gel layers of the small ends. Other processes such as cell migration, cell multiplication and growth also play a role in shaping the brain. Increase in lumen size by hydrostatic pressure of fluid secreted into it may also play a part.

Optic vesicle evagination

A glance at a section through the optic vesicle (fig. 16) is sufficient to indicate that the same mechanical principle is involved here as in neural tube invagination. Cell boundaries are not distinct but it is evident that the ends of the radially arranged cells facing the concave surface are smaller than the outer ones. Most of the nuclei have been squeezed towards the convex surface along with endoplasm.

Optic cup invagination

Optic cup invagination (fig. 17) seems to be due to a reversal of the contractile tension of the gel layers of the cell ends facing the cup cavity. Here the ends are smaller than at the convex surface. They are especially narrow at the abrupt bend. The cells have a new radial arrangement.

Lens invagination

The lens arises from the inner layer of the ectoderm. Figure 17 indicates that invagination is due to the contraction of cell ends facing the concavity. Contraction of the concave surface area has crumpled the overlying outer layer of the ectoderm. This indicates that the contracting cell ends responsible for invagination, exert considerable force.

Ear vesicle invagination

The ear vesicle, like the lens, arises from the deep layer of the ectoderm. It is evident from figure 18 that invagination is due to the contraction of the ends of the cells facing the concave surface. One can see at the center of the concave surface small pigmented ends. The overlying outer layer of the ectoderm is slightly crumpled by the contraction of the invaginating surface.

Nasal pit invagination

Nasal pit invagination (fig. 19) involves both layers of the ectoderm. There is excessive contraction of the superficial ends of the cells facing the concave surface into slender pigmented necks.

Blastopore invagination

Blastopore invagination will be considered in more detail elsewhere. It is irregular and rudimentary. It plays a minor role in gastrulation and forms only a small part of the primitive archenteron.

DISCUSSION

Various theories of invagination have been advanced from time to time.

Holtfreter ('44a) has suggested that the surface may be pulled inward by migrating cells that remain attached to the surface by long necks as in the blastopore region of amphibia. Long neck flask cells occur in other invaginations but they remain in the invagination plate and obviously could not pull the concave side inward and at the same time cause the convex surface to bulge. Most invaginations begin before cells on the concave side are elongated into flask cells with long necks.

Glaser's ('14, '16) idea that folding of the neural plate is due to cell enlargement and hydration has been refuted by Brown, Hamburger and Schmitt ('41) and by Gillette ('44).

They find no evidence of swelling. Increase in size, by swelling of the deep ends could not possibly produce folding or invagination. It would have just the opposite effect.

The idea that differential surface tension forces may be responsible for invagination is untenable. The superficial gel layer is the most superficial part of a cell. It is the surface layer. The superficial aspect of the gel layer is firm, tough, and pliable and more viscous than its deep aspect where it merges into endoplasm. Cells do not have a fluid surface layer with molecular or bimolecular films.

The factors responsible for superficial gelation of cytoplasm and for local changes of its contractile tension that result in invagination and inward migration of cells from the invaginating surface are unknown. A clear explanation as to just what happens when cytoplasm changes from sol to gel or gel to sol is not available. Hydration and dehydration and decrease and increase of the linkages between long protein molecules have been suggested as possibilities.

Cytoplasm has no absolute viscosity and is probably continually changing. Reversible changes can be induced by various agents, acids, alkalies, carbon dioxide, oxygen, potassium, magnesium, sodium, etc., but to none of them can be ascribed, with any degree of certainty, the changes of gel layer viscosity responsible for invagination.

There are indications that something in the external environment, perhaps oxygen, may produce a basic superficial gelation and that modifications of the internal or external environment may increase or decrease the thickness and/or viscosity of this basic gel layer. Gel layer is always in a delicate state of unbalance and responds by local changes of contractile tension to infinitestimal stimuli in either external or internal environments.

The 2 surfaces of an epithelial membrane are exposed to somewhat different environments and as differentiation proceeds the exposed gel layers on the 2 surfaces may react differently. Such an environmental difference does not explain why pieces of neural plate embedded below the surface curl

into tubes. This leads one to suspect that cells in an epithelial membrane become polarized and that this may account for the differences of contractile tension on the 2 surfaces.

The thickness, viscosity and contractile tension of gel layers presumably vary with the type of cytoplasm and with its metabolic condition. During cytoplasmogenesis changes probably occur in the contractile tensions exerted by gel layers of different types of cells when they attain certain stages of differentiation. Thus during development the behavior of cells (changes of form, migration, invagination, etc.), may indicate cytoplasmic differentiations not appreciable by any other known criterion.

A complete understanding of the factors responsible for the superficial gelation and for local changes of it will advance markedly our knowledge of cells, their metabolism and development.

SUMMARY

Invagination plays an important role in the development of a variety of organs.

The essential factors for invagination are an increase or a decrease of the contractile tension of the superficial gel layers on one side of a group of adherent epithelial cells that offer some resistance to distortion. Invagination occurs on the side of the greater tension.

The peculiar characteristics of each invaginating organ are determined by a great variety of modifying factors.

A model illustrating the mechanics of invagination and examples of it are presented.

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LIMB ABLATION EXPERIMENTS ON THE EMBRYONIC CHICK AND ITS EFFECT AS OBSERVED ON THE MATURE NERVOUS SYSTEM [1]

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SEVEN FIGURES

In a series of limb extirpation experiments Hamburger and Keefe ('44) found the limb periphery has little or no influence on proliferation and the resulting cell number in the chick spinal cord. Cell counts in Detwiler's experiments on Amblystoma in which extensive peripheral changes were made by limb ablation ('24), limb transplantation ('36) and somite removal ('29) also indicate that cell numbers in the spinal cord are not altered by peripheral changes. Hypoplastic and hyperplastic changes in cell groups and columns of the spinal cord as a result of peripheral changes seem therefore to be phenomena of cellular differentiation. Motor cells which are present on the limb side of an ablation experiment are represented by undifferentiated cells on the opposite side.

The effect of the limb periphery on differentiation of the posterior column, intermediate gray substance, anterior lateral column and spinal ganglia have been previously studied in the chick (Shorey, '08; Hamburger, '34, '39, '44; Bueker, '43, '45a, '45b). Most of these experiments were terminated at 9–11 days incubation. Cross sections of lumbosacral spinal cords terminated at this time seem to show some difference in cell size in the intermediate gray matter. Large multipolar

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cells, some of which are in or near the position of Clark's column in the mammalian cord are significantly reduced in size as a result of limb extirpation. I suggested (p. 355, Bueker, '45) that the differentiation of the spinocerebellar system was probably involved.

In the present investigation the purpose was primarily to obtain maximal hypoplastic changes in the intermediate gray matter and other regions of the spinal cord by terminating radical limb extirpation experiments after neurological maturity. Stultz ('42) was able to demonstrate a hypoplasia of motor cells in limb ablations on embryonic stages of Amblystoma when animals were sacrificed at or near the time of metamorphosis. Similar experiments by Detwiler ('24, '29, '36) in the animals sacrificed earlier gave negative results. Although maximal changes were observed in the lateral motor column (a hypoplasia of 90%) of hind limb extirpations on 48–60-hour chick embryos terminated at 9–11 days total incubation, it is believed, that an extension of time to 3 months after hatching would enable one to observe increased differences in the posterior column and the intermediate gray matter, especially in such cells as are homologous to those in Clark's column of the mammalian spinal cord.

The extent of limb removal must also be considered in obtaining a maximal hypoplasia in corresponding sensory and motor centers of the spinal cord. A linear relationship has been experimentally demonstrated between the amount of limb removal and the degree of hypoplasia for the lateral motor column in the chick (Hamburger, '34). However, Piatt ('45) has clearly shown that the hyperplasia and hypoplasia in the mesencephalic nucleus of Amblystoma is not a simple linear function of peripheral increase and decrease. In the present investigation, however, it would seem necessary to completely remove the hind limb periphery to obtain an optimum hypoplasia of all the responsive neuro-cellular elements.

Since peripheral nerves serve as mediators of causal factors in the periphery, transmitting them to the cell body and

dendrites and thus causing inductive changes on the surrounding indifferent neuroblasts, it is necessary to remove the limb primordium before it becomes innervated. Severing peripheral nerves subsequent to innervation may give sufficient time for some of the inductive changes to become established.

Possible retrograde and transneuronal degenerative changes which follow the sectioning of peripheral nerves are also objectionable and would defeat the purpose of this investigation. Recent experiments by Romanes ('46) indicate that this occurs in the lumbosacral spinal cords of newborn mice after limb amputation, crushing, or sectioning the sciatic nerve. It would seem impossible to eliminate this factor in limb amputations on early fetal stages such as were done on the sheep (Barron, '45) and on the rat (Hall and Schneiderhan, '44).

In using the chick as an experimental animal most of the objectionable features which have been mentioned can be avoided. Limb primordia are removed according to a method previously described (Bueker, '43). This method has several advantages: (1) the limb field can completely be removed before it becomes innervated, (2) the spinal cord can be subdivided into sensory and motor centers clearly set apart from each other, (3) experiments may be terminated at any time after the operation.

METHOD

General methods for limb removal have been described (Hamburger, '42). Forty-eight to 60-hour incubated eggs are candled and the position of the embryo marked. A small rectangular hole is cut in the shell immediately above the embryo. The egg is gently rocked as the saline is absorbed. Gradually a space appears between the shell and vitelline membranes. The shell membrane may now be removed.

Agar stamps with neutral red (Hamburger, '42) are used to stain the vitelline membrane after which it is removed. Somites and the site of the hind limb field are stained and the

somites counted. At this time the hind limb primordium, which is removed with a glass needle is a restricted area in the somatopleure immediately lateral to somites 24–32, having the width of approximately 2 somites. Limb forming potencies of the chick blastoderm from the primitive streak to 15-somite stages have been experimentally determined (Rudnick, '45). This information was valuable in using embryos in which somites 24–32 were not completely separated.

Of 30 operated animals only 5 survived hatching. Two of these died during the first month; the remaining 3 (A-6, A-7, A19, figs. 4, 5, 6) which are here reported were sacrificed at the end of the third month. In removing the limb field some of the somatopleure which was destined to form the body wall was usually included. As a result the body wall was often herniated or the viscera ectopic. Failure of closure of the abdominal wall accounted for a high mortality at or near the time of hatching.

The central nervous system and lumbosacral plexus were exposed and removed. The upper one-half of segment 25 was fixed in alcohol; the remainder in Bouin's fluid. Spinal ganglia and the proximal stumps of lumbosacral peripheral nerves were left in place in the intervertebral foramina. Some of this material was later decalcified and sectioned. Segments 19–30 inclusive were imbedded and serially sectioned at 10 μ. A few sections of the brain stem, segments 9 and 15 were also prepared.

To give expression to volumetric differences between the 2 sides of the spinal cord, 5 sections were selected at random from each segment (19–30) of each case (A-6, A-7, and A-19). Cross sections were projected at magnifications of 25–30 and drawn. The anterior fissure, neural canal, and dorsal septum were used to divide cross sections into right and left halves. Dorsal root fibers were used to separate the posterior from the antero-lateral funiculus. In segments 19–30 the gray matter was subdivided into intermediate gray, posterior column, and motor columns (fig. 2). These areas were measured with a planimeter. Since the degree of reduction in

volume had a similar pattern in each of the 3 cases, the amount of reduction in per cent for each spinal cord subdivision through segments 19–30 was calculated by comparing the total of 15 measurements on the 2 sides. Results of these measurements are summarized in figure 3.

To estimate the effect of limb ablation on cell number 50 sections stained with cresyl violet (30 from A-6; 20 from A-7) were selected from segment 25. Camera lucida drawings of the posterior column, intermediate gray matter, mesial and lateral motor columns were made on squared paper. Neurons in which nuclei were visible were marked on the drawings and then counted. Cells in the mesial motor column were not counted and will be considered later in a separate study. To correlate changes in cell number with volume, subdivisions in which cells were counted were also determined for area. The areas of the dorsal funiculus and the antero-lateral funiculus of the above sections were also measured.

RESULTS

An estimation of the extent of limb removal of the 3 cases reported was made from gross dissections of the pelvic region. Success of limb removal was such that in each case the head of the femur, acetabulum, and most of the ilium was missing. Portions of the ischium with some of its attached musculature remained. Since the degree of limb removal was approximately the same in each of the 3 cases, individual descriptions are omitted.

Peripheral nerves on the ablated side were reduced $\frac{1}{3}$ to $\frac{1}{4}$ the size of those located contralaterally (fig. 1). Spinal ganglia were reduced approximately $\frac{1}{2}$ to $\frac{2}{3}$. In 2 cases (A-6, A-7) peripheral nerves 22–24 did not anastomose to form the femoral nerve. This may be accounted for by the complete absence of the ilium in these levels. From this observation and others made previously in limb extirpation experiments terminated at 9–11 days incubation one may infer that the innominate primordium is essential for moulding and maintaining the lumbosacral plexus.

Spinal cord segments (22–30) are strikingly asymmetrical. This asymmetry becomes less through segments 19–21. Except for a deficiency in myelinated fibers near the dorsal septum on the limb ablated side cross sections through the brain stem, segments 9 and 15 appear normal. The location of this deficiency corresponds to the position of the long

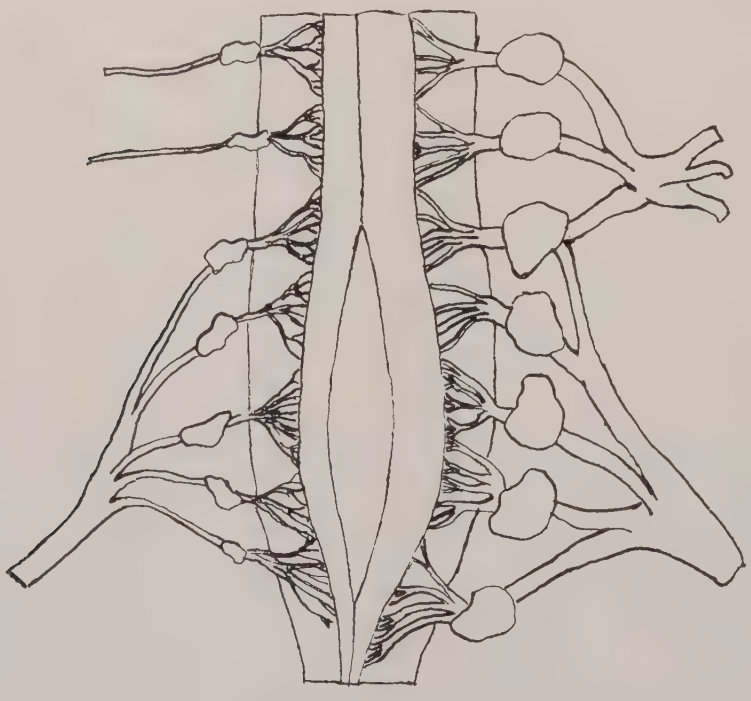


Fig. 1 A-17, left limb primordium removed. Dorsal view of the spinal cord and peripheral nerves showing the reduction on the left side. Experiment terminated the third month after hatching.

ascending tracts in the dorsal funiculus as demonstrated experimentally by Friedländer (1898) in degeneration experiments on the pigeon. No abnormalities are found in the area occupied by spinocerebellar fibers. We should like to suggest that transections of the spinal cord above the lumbosacral level and allowing sufficient time for the long fiber tracts to degenerate would have given conclusive information on the

extent of reduction of ascending tracts. This was not done at this time because of an insufficient number of animals.

Cell counts of the lateral motor column in 50 sections were 961 on the limb side and 95 contralaterally; a reduction of

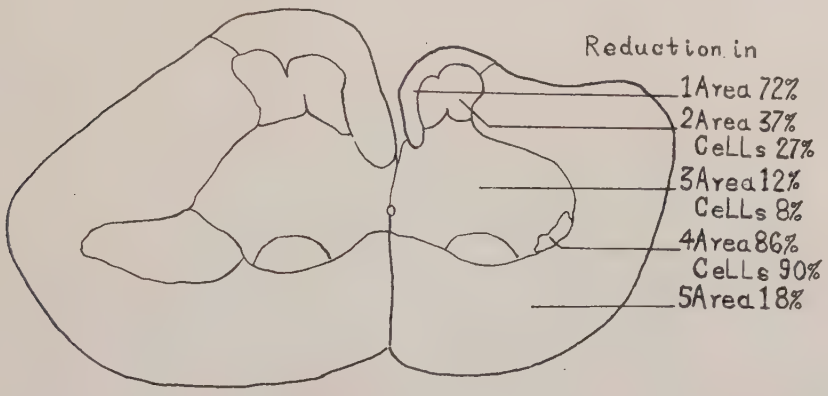


Fig. 2 A-5, right limb primordium removed. Diagram of a cross section through segment 25 showing the reduction in area and cells for the various subdivisions of the spinal cord. Percentage reduction of each area is based on a total of measurements in 50 cross sections.

91% (fig. 2). The reduction in area for this column varied approximately 80–90% in segments 23–29; 30–43% in segments 22 and 30 (fig. 3).

The dorsal funiculus is reduced 72–77% in segments 24–27; 50–58% in segments 22, 23, 28, and 29 (fig. 3). In segments 19, 20, and 21 the reduction varied from 18 to 28%. Above segment 19 this portion, except for a small region near the dorsal septum, again becomes symmetrical. Measurements in segment 15 gave reductions of -3 to $+5$%.

Cell counts (every fourth section) of spinal ganglia 25–28 of experiment, A-6, totaled 6279 on the amputated side as compared to 12,512 contralaterally; a decrease of approximately 50%. This reduction is typical for the lumbosacral spinal ganglia of the 3 cases. The hypoplasia in ganglion cells, however, is not as high as the difference in volume of the dorsal funiculus. If one could assume that this funiculus is composed largely of proprioceptive fibers, and that the cells

of origin in the spinal ganglia undergo a higher degree of hypoplasia than other ganglion cells, then this discrepancy could partly be explained.

The posterior column is reduced 38–45% in segments 23–29; 20–25% in segments 20, 21, 22, and 30 on the experimental side

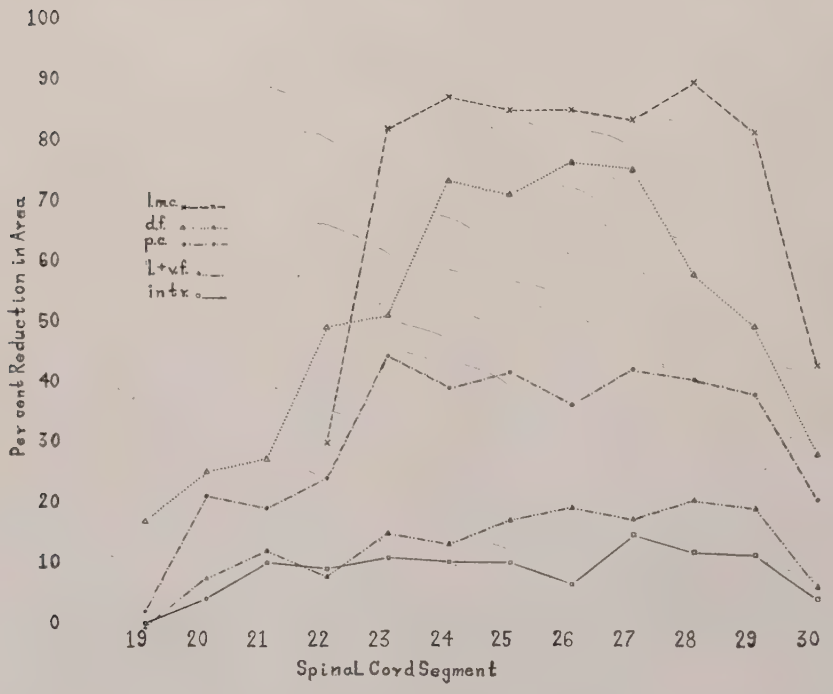
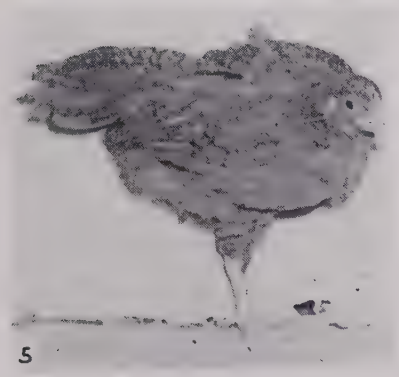


Fig. 3 Per cent reduction in volume of spinal cord subdivisions (l.m.c., lateral motor column; d.f., dorsal funiculus; p.c., posterior column; 1 + v.f., antero-lateral funiculus; intr., intermediate gray substance) based on an average of 3 limb extirpation experiments A-5, A-6, A-17 through segments 19–30; cellular hypoplasia of the lateral motor column through segments 22–30.

of the cord (fig. 3). Cell counts of this area (50 sections) were 14,026 on the limb side and 10,351 on the opposite side; a reduction of 27% (fig. 2). Volume reduction is due to: (1) a decrease in cell number and cell size (figs. 2, 7), (2) a reduction in number of afferent fibers from the dorsal root and collaterals from the posterior funiculus. The discrepancy be-

tween the reduction in volume and cell number observed on the limb ablated side of the spinal cord (fig. 2) may be accounted for by the difference in density of afferent fibers and degree of differentiation.



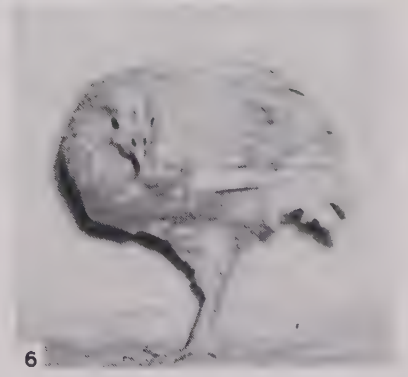


Fig. 4 A-5, right limb primordium removed at 58 hours incubation. Three months after hatching; normal animal of same age on the right.

Fig. 5 A-6, right limb primordium removed at 50 hours incubation. Three months after hatching.

Fig. 6 A-17, left limb primordium removed at 51 hours incubation. Three months after hatching.

A volume reduction of 8–16% in the intermediate gray matter on the limb ablated side of the spinal cord was observed in segments 21–29; a cell reduction of 8% in segment 25 (figs. 2, 3, 7). As in the posterior column the density of afferent fibers entering this region is reduced, which is associated with an inhibition of cellular differentiation and a decrease in cell number. Large cells in and around the vicinity of Clark's column as represented in mammalian spinal cord were counted (every fourth section) in 1 case, A-6, through

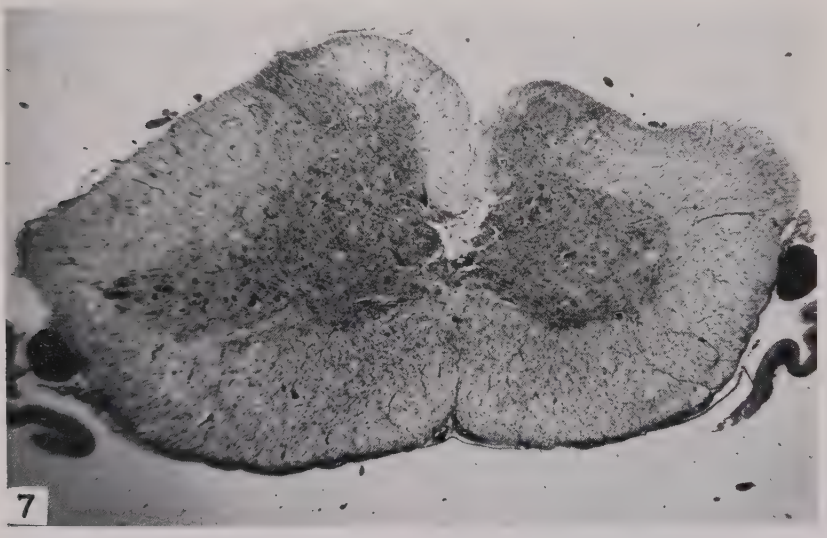


Fig. 7 A-5, right limb primordium removed. Lumbosacral spinal cord; cross section through segment 25.

segments 22–25. On the limb extirpated side of the spinal cord 775 cells were counted as compared to 1024 on the opposite side; a reduction of 24%. The position of the cells which were counted are similar to those described as Clark's column in the Ostrich and are said to give rise to certain of the spino-cerebellar fascicles (Streeter, '04).

The antero-lateral funiculus is decreased in size as a result of limb ablation 9–21% in segments 20–29; 18% in segment 25 (figs. 2, 3, 7). This decrease is linked with the cellular hypo-

plasia in the intermediate gray matter, and a corresponding decrease in the number of efferent fibers extending from these cells into the lateral funiculus.

Assymetry in volume extends above the lumbosacral segments of the spinal cord, through segments 19–21 (fig. 3). On the limb ablated side of the spinal cord the dorsal funiculus is considerably reduced in these segments, and one can observe a decrease in the density of afferent fibers which enter the posterior column and intermediate gray matter. With this decrease in fibers is associated a lower degree of cellular differentiation.

DISCUSSION

1. A comparison of results with radical limb extirpation experiments terminated at 9–11 days total incubation

Since the mechanism of peripheral interaction seems definitely to be linked with cellular differentiation it would seem possible that maximal changes in differentiation in the nervous system as a result of peripheral alterations could be observed only after animals are mature. It was observed previously that the degree of differentiation of neurons in the intermediate gray matter was less on the limb ablated side of the spinal cord if animals sacrificed at 9–11 days total incubation (Bueker, '43). The purpose of this investigation was primarily to observe volumetric and cellular changes in the intermediate gray matter of lumbosacral spinal cords in radical hind limb extirpation experiments after allowing sufficient time for animals to reach maturity, and also to examine upper levels of the spinal cord for possible defects.

Since the method of removing the hind limb primordium and its effectiveness as judged by complete absence of the lower extremity and most of the pelvic girdle was the same in present experiments as those reported earlier (Bueker, '43, '45a) a comparison of results is possible. Such a comparison will bring out those changes occurring as a result of

limb ablation between the ninth day total incubation and the third month after hatching.

Cell counts and volume determinations (figs. 2, 3) of the lateral motor column in the lumbosacral cord indicate a cellular hypoplasia of approximately 90%, which is essentially the same as for experiments terminated at 9–11 days total incubation (Bueker, '43). Cell counts of lumbosacral spinal ganglia 23–29 (every fourth section) of the host, Tr 28, totaled 9994 on the experimental side and 18482 contralaterally. In this experiment (Tr 28) which was previously reported (Bueker, '43) a spinal cord graft inserted between the host cord and the right hind limb field blocked the outgrowth of host nerves; giving results similar to a limb extirpation experiment. The hypoplasia of ganglion cells of 46% gives some indication of the influence of limb periphery at 9 days incubation, when this experiment was terminated. The hypoplasia of lumbosacral spinal ganglia at 3 months after hatching was also approximately 50%.

Volumetric studies of the posterior column in the lumbosacral cord give a reduction of 34% in limb ablation experiments terminated at 9–11 days incubation (Bueker, '45b), and approximately 40% in the present experiments (figs. 2, 3). In wing extirpation experiments a cellular hypoplasia as high as 21% was obtained at 8–9 days incubation (Hamburger, '34); cell counts of the posterior column in the lumbosacral spinal cord at 9–11 days incubation are not available for comparison. Apparently the hypoplasia in the posterior column is somewhat higher as judged by volume changes at 3 months after hatching than at 9–11 days total incubation.

Although the degree of reduction in ganglion cells, lateral motor cells and the posterior column remains relatively constant during the interval between 9–11 days incubation to the third month after hatching, this does not hold for the intermediate gray matter. In limb extirpation experiments terminated at 9–11 days total incubation, the volume and cell number in this portion of the lumbosacral cord is approximately equal on the 2 sides (Hamburger, '34, '44; Bueker,

'45b). In the present experiments there is a reduction in volume for this region of the lumbosacral cord of approximately 12% and a cellular hypoplasia of 8% in segment 25 (figs. 2, 3) on the limb ablated side. One can also observe a decrease in the density of collaterals from the dorsal funiculus on the experimental side and a corresponding decrease in cell size.

Since the density of afferent fibers from the dorsal funiculus and efferent fibers extending into the antero-lateral funiculus is less in the intermediate gray matter on the limb ablated side of the spinal cord than contralaterally, volume determinations of the antero-lateral and dorsal funiculi should also be less than on the normal side. At 9–11 days incubation the reduction of the dorsal funiculus through segments 22–30 averaged 29% (Bueker, '45b) as a result of limb ablation, and approximately 60% the third month after hatching (figs. 2, 3). This increased reduction in the dorsal funiculus at the end of the third month is due to an accumulation of short afferent fibers on the normal side and cannot be explained by changes in the number ganglion cells. Experimental evidence indicates that most of the fibers in the dorsal funiculus end in the gray matter within a few segments of their entrance into the cord; only a few ascend as far as the medulla (Friedländer, 1898).

Positions of efferent motor roots vary considerably and a division of the anterior and lateral funiculus is somewhat arbitrary, however, this division was made in reconstructions of the lumbosacral cord of limb extirpation cases terminated at 9–11 days and reductions for the anterior and lateral funiculus were 15% and 18% respectively (Bueker, '45b). At the end of the third month the total reduction for both funiculi, referred to as the antero-lateral, was 16–18% through segments 22–29 (figs. 2, 3). Bilateral asymmetry in this part of the white matter undergoes little change during this interval. Inasmuch as the difference in degree of cellular differentiation in the intermediate gray matter was greater at the end of the third month after hatching than at 9 days total incubation, one would also expect a greater difference in the antero-

lateral funiculus at the end of the third month. The methods used for studying volume differences were not accurate enough to give significant data.

According to Kappers, Huber and Crosby ('36) spinocerebellar tract in birds arises from all levels of the cord and receives a considerable component from the lumbar region. Since the cells of origin of spinocerebellar fascicles are considerably reduced in size and number in the lumbosacral segments as a result of limb ablation one would expect a corresponding reduction of this tract in the antero-lateral funiculus. Degeneration of ascending tracts as a result of transecting spinal cords above the lumbosacral segments of mature limb extirpation cases should give added evidence as to the extent of involvement of this system.

2. Mechanism of interaction with the non-neural periphery

Coghill ('29) has described the intrinsic basic pattern of proliferation and development in the central nervous system of Amblystoma during early stages. Such a pattern is also present in the chick and experimental results give indications that it is not influenced by the non-neural periphery (Hamburger, '44). Presumably the expression of this intrinsic mitotic pattern is responsible for the production of indifferent neuroblasts which will not differentiate unless they come under the influence of developing non-neural sensory and muscular structures.

To explain the causal relations between the periphery and the differentiation of corresponding sensory and motor centers of the spinal cord it is convenient to combine the concepts of selective fasciculation (Weiss, '41), induction from the cell body (Hamburger, '34, '44) and the stimulating effect of outgrowing fibers on the differentiation of surrounding neuroblasts (Barron, '45).

In applying the theory of selective fasciculation (Weiss, '41) to the initial changes which occur during the outgrowth of pioneer nerve fibers of primary sensory and motor neurons

it is necessary to assume that the periphery imposes biochemical changes on some of the fibers causing their surface properties to change so that other nerves in the vicinity will be attracted to them and thus be instrumental in forming a nerve bundle. The initial change in these fibers is peripherally imposed and occurs before motor end plates and muscle spindles are formed (Hamburger, '44). These changes are then projected from the peripheral fibers to the cell bodies (Hamburger, '44) and dendrites (Barron, '43, '45) so that inductive properties responsible for stimulating the differentiation of surrounding neuroblasts are present in the cell body or any of its processes.

Hamburger ('44) recognizes 3 different components in the mechanism of peripheral control: (1) the setting up of a stimulus by a peripheral field to be innervated, (2) changes occurring in the primary neuron as a result of this stimulus, (3) the inductive effect of the primary neurons on indifferent cells. Properties of the peripheral field in which the stimulus resides remain largely unknown. It has been found, however, that the stimulus from the hind limb field of chick embryos is not species specific for the development of the lateral motor column. Lumbosacral spinal cord grafts from the guinea hen will differentiate a lateral motor column under the influence of the chick hind limb (Bueker, '45). Neither does a point for point organ specificity exist. That is, the brachial spinal cord segments when transplanted so as to come under the influence of the hind limb will differentiate an excellent motor column (p. 359, Bueker, '45).

3. Significance of changes observed in the intermediate gray matter

The most striking peripherally induced changes as a result of limb ablation are complete at the end of 9 days total incubation in the chick. However, during the interval between 9–11 days incubation and the third month after hatching there is a continued increase of the dorsal funiculus on the limb side of the spinal cord and a corresponding increase in the

density of collaterals extending from this funiculus into the intermediate gray matter. During this interval the afferent fibers ramify among the indifferent cells. According to Barron ('45) these fibers are responsible for causing cell differentiation. Although peripheral changes in the intermediate gray matter do not present a picture which is as prominent as observed in other parts of the spinal cord, they may be equally important in the development of the neural mechanism essential for limb function. The reduction of cells and volume in this region of the spinal cord is somewhat lower than that obtained by Barron ('45) in limb ablation experiments on fetal sheep.

SUMMARY

Radical limb extirpation experiments were done on the 48–60-hour chick embryo. These experiments were terminated after the animals were mature (3 months after hatching). Volumetric and cellular studies of spinal cord subdivisions (fig. 2) through spinal cord segments (22–30) and cellular studies through spinal ganglia 25–29 on the limb ablated side as compared to similar studies contralaterally gave the following results: (1) the percent reduction of the lateral motor column was approximately 90% which is similar to results in experiments terminated at 9–11 days total incubation (Bueker, '43) (2) the cellular hypoplasia in spinal ganglia 25–29 of 50% is also similar to results obtained at 9–11 days incubation; (3) the reduction in volume (segments 22–30) and cell number (segment 25) of the posterior column was 40% and 27% respectively, reduction in volume was somewhat higher than 9–11-day incubation experiments; (4) the posterior funiculus is reduced 60% in volume as compared to 27% at 9–11 days incubation; (5) reduction in cell number (segment 25) and volume (segments 22–30) was 8% and 12% respectively in the intermediate gray matter; at 9–11 days incubation this region of the cord, except for the nucleus dorsalis, is approximately symmetrical.

Asymmetry in the posterior column and intermediate gray matter extends through segments 19–21. This is associated

with an increase in density of collateral fibers from the dorsal funiculus on the limb side and a decrease on the opposite side. Except for a deficiency in a small area near the dorsal septum of the posterior funiculus on the limb ablated side, cross sections in the brachial level and upward appeared normal.

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STUDIES ON THE LIGAMENTS OF THE OVIDUCT IN THE DOMESTIC FOWL

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SIXTEEN FIGURES

INTRODUCTION

It is well known that the oviduct in the domestic fowl is held in position by 2 ligaments, 1 ventral and the other dorsal, which form very conspicuous structures in an adult hen. Unfortunately the developmental history of these structures has received scant attention from the previous workers.

In the present paper an attempt has been made to investigate the embryonic development of the ligaments. Attention has been paid to the morphology and histology of the ligaments in immature chicks and their growth and differentiation with the approach of sexual maturity. An opportunity has also been taken in the course of this study to throw some light on the function of the ligaments.

REVIEW OF THE LITERATURE

Curtis ('10) was the first to give a comprehensive account of the morphology of the ligaments in adult hens. She also gave a very brief account of the morphology of the ligaments in young chicks and further suggested that the tubal ridge (vide Lillie, '27) is responsible for the formation of the ventral

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ligament. Hewitt ('39) also described the morphology of the ligaments in adult hens.

Curtis ('10) and later on Kaupp ('18) pointed out that the oviduct and its attached ligaments elongate considerably with the onset of sexual maturity.

Curtis ('10) showed that the ligaments terminate anteriorly in such a manner that they form a sort of "Pocket" which guides the egg into the funnel portion of the oviduct. She also suggested that the musculature of the ligaments helps, to a great extent, in maintaining the normal peristalsis of the oviduct. Asmundson ('31) pointed out that the shape of the egg is determined mostly by the activity of the muscles on the wall of the isthmus and the uterus and the ligaments exert no influence on the egg shape. Hewitt ('39), on the other hand, observed that the ligaments influence the egg shape and recently Harper and Marble ('45) subscribed to his view.

MATERIAL AND METHOD

Eggs from the pure-bred Brown Leghorn hens of the Institute flock were incubated in an electric "Petersime" incubator. The embryos among the female line were sorted out by the careful examination of their gonads (Greenwood, '25) and sections were prepared by the usual method.

For histological study, the entire oviduct with its attached ligaments was dissected out and fixed in toto. Ten per cent formol-saline proved to be useful for the latter purpose. After the usual process of dehydration and clearing small pieces of oviduct with the ligaments in situ were embedded in paraffin and transverse sections were prepared. The latter were stained with Mallory's triple stain or Ehrlich's haematoxylin followed by alcoholic eosin. For the study of collagenous fibers small pieces of the ligaments from fresh unfixed material were stained with methylene blue and mounted in glycerine.

Only the development and differentiation of left ventral and left dorsal ligaments were studied.

OBSERVATIONS

A. Embryonic development of the ligaments

The method of description of the embryonic stages followed here is the same as in a previous paper (Kar, '47).

a. The ventral ligament. Stage I (8 days old). The mullerian duct is seen as an epithelial tube surrounded by a thick outer tunic of concentrically arranged mesenchyme cells. The thickened epithelium of the tubal ridge appears flat and similar to the adjacent peritoneal epithelium. On the mid-ventral surface of the mullerian duct a swelling is observed which extends from the posterior end of the non-sexual division of the urinogenital ridge for a short distance only caudad. In transverse section this swelling appears as the mid-ventral bulging of the peritoneal epithelium covering the urinogenital ridge and is loosely filled with mesenchyme cells (fig. 1). It measures about 3 microns in diameter.

Stage II (9 days old). At this stage the swelling on the mid-ventral surface of the mullerian duct is seen up to the ostium region cephalad and down to the cloacal region caudad. In the cranial region the mesenchymatous cells from the outer tunic of the müllerian duct migrate into the swelling and, as a result, it becomes closely packed with mesenchyme cells (fig 2). In the caudal region, however, no cell migration is observed and the swelling remains loosely packed with mesenchyme cells as in the previous stage.

ABBREVIATIONS

b.v., blood vessel.
c., constriction.
c.f., collagenous fibers.
d.a., dorsal aorta.
d.b., dorsal body wall.
d.v., diverticulum-like projection of the dorsal body wall.
d.l., dorsal ligament.
fl., flexure.
m.d., mullerian duct.
m.b., blocks of muscular tissue.
m.s., mesenchymatous strip.
m.t., muscle strands.
m., muscular tissue.
mc., mesenchymatous cells.
n.c., notochord.
o.d., oviduct.
pb.u., posterior blind sac of the uterus.
s.k., slight kinking.
u., ureter
ut., uterus.
v.l., ventral ligament.
v., vagina.
w.d., wolffian duct.

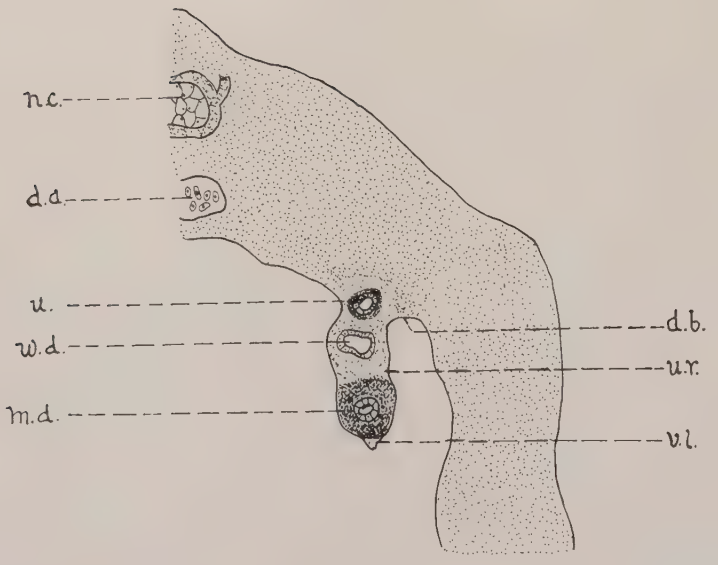

Fig. 1 Transverse section through the caudal portion of the urinogenital ridge of an 8-day-old female chick embryo showing the anlage of the ventral ligament (× 25).

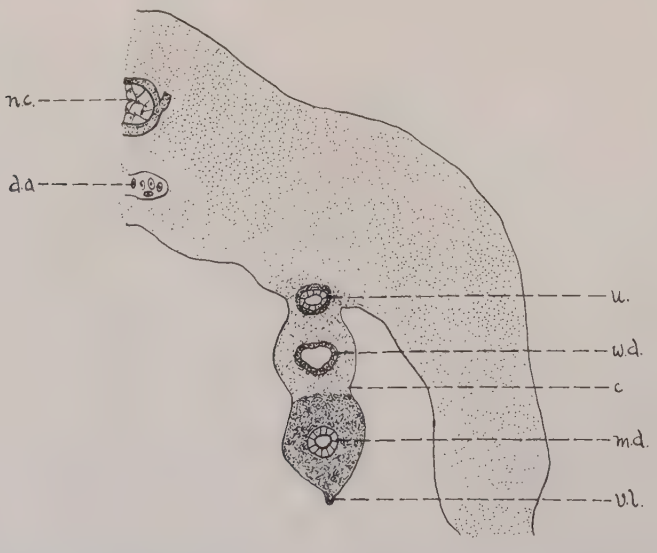

Fig. 2 Transverse section through the caudal portion of the urinogenital ridge of a 9-day-old female chick embryo showing the anlage of the ventral ligament (× 30).

Stages III and IV (10 and 11 days old). The process of cell migration from the outer tunic of the mullerian duct is extended down to the caudal region and, as a result, in this area also the swelling becomes closely packed with mesenchyme cells. In transverse section it appears as the mid-ventral protuberance from the outer mesenchymatous tunic of the mullerian duct covered by the peritoneal epithelium of the urinogenital ridge (figs. 3 and 8).

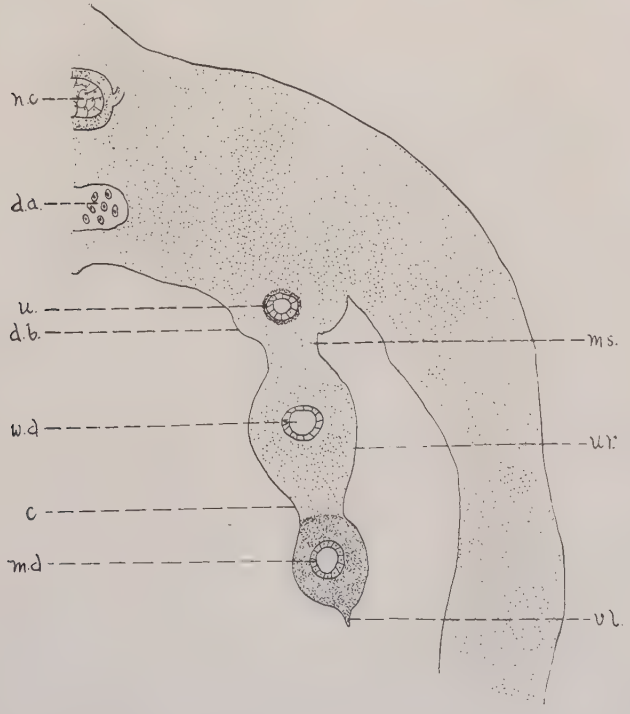

Fig. 3 Transverse section through the caudal portion of the urinogenital ridge of a 10-day-old female chick embryo (× 35). For further explanation see the text.

Stage V (12 days old). The left mullerian duct has differentiated into the ostium, the magnum and the primordium of the shell-gland. The mid-ventral swelling of the mullerian duct or the ventral ligament exhibits considerable increase in diameter and in the cephalic region its free end appears some-

what curved (fig. 9). Near the caudal region of the primordium of the shell gland the future ventral ligament appears as a spherical cord.

Stages VI and VII (15 and 17 days old). The ventral ligament is supplied with blood vessels and blocks of muscular tissue of the plain type and seen near the free end (fig 12). Patches of muscular tissue continues with that of the mullerian duct are also seen along the 2 lateral margins of the ligament (fig. 4). Collagenous fibers are seen throughout its

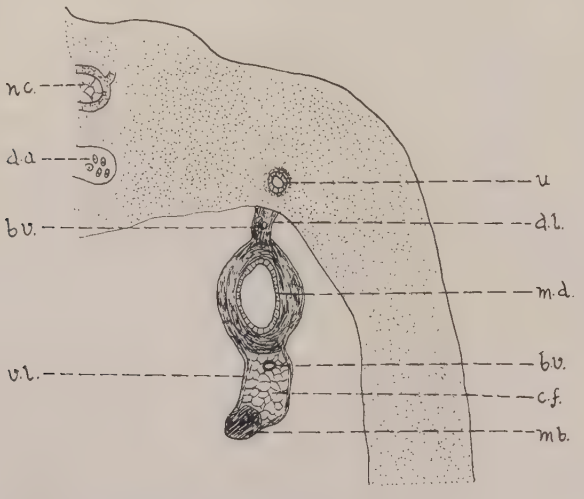

Fig. 4 Transverse section through the magnum region of the mullerian duct of a 17-day-old female chick embryo showing the ligaments (× 30).

extent except in the shell gland region where it appears only a muscular cord without any collagenous fibers.

Stages VIII and IX (20 and 21 days old). The free ventral margin of the ligament appears like a muscular cord and the 2 lateral margins are marked by the presence of thin patches of muscle which are continued round the mullerian duct. The rest of the ligament, however, is filled with collagenous fibers. In the caudal region of the shell gland the ventral ligament is a muscular cord without any collagenous fibers as in the previous stages (fig. 13).

b. The dorsal ligament. Stage I (8 days old). Except in the cloacal region, the mullerian duct occupies a ventral position to the wolffian duct in the caudal portion of the urinogenital ridge and is surrounded by a thick outer tunic of mesenchyme cells. The wolffian duct is surrounded by a rather thin layer of mesenchyme cells (fig. 1). The ureter is situated close to the junction of the urinogenital ridge with the dorsal body wall. The anterior end of the wolffian body and the wolffian

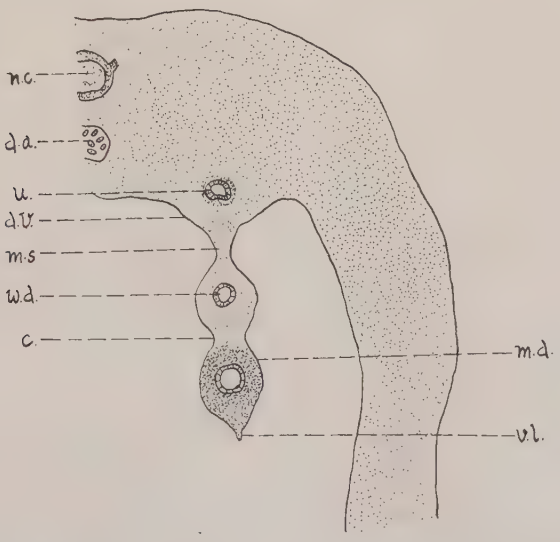

Fig. 5 Transverse section through the caudal region of the urinogenital ridge of a 9-day-old female chick embryo in the region of the cloaca (× 30).

duct have degenerated and the ostium region of the mullerian duct remains attached to the dorsal body wall by a strip of mesenchyme (fig. 10). Further caudad i.e. in the remaining sexual and the whole of the non-sexual portions of the wolffian body (urinogenital ridge) the mullerian duct occupies its usual position in relation to the wolffian duct.

Stage II (9 days old). In the caudal portion of the urinogenital ridge (Kar, '47) a slight constriction is observed in its middle region (fig. 2). In the cloacal region the portion of the urinogenital ridge dorsal to the wolffian duct also becomes

constricted and appears as a narrow mesenchymatous strip which remains attached to a diverticulum-like portion of the dorsal body wall (fig. 5). The ureter is situated in the latter region and is surrounded by a thick mesenchymatous outer tunic. In the ostium region the mullerian duct remains attached to the dorsal body wall by the mesenchymatous strip referred to in the previous stage. In the entire cephalic portion of the urinogenital ridge (wolffian body) the wolffian duct

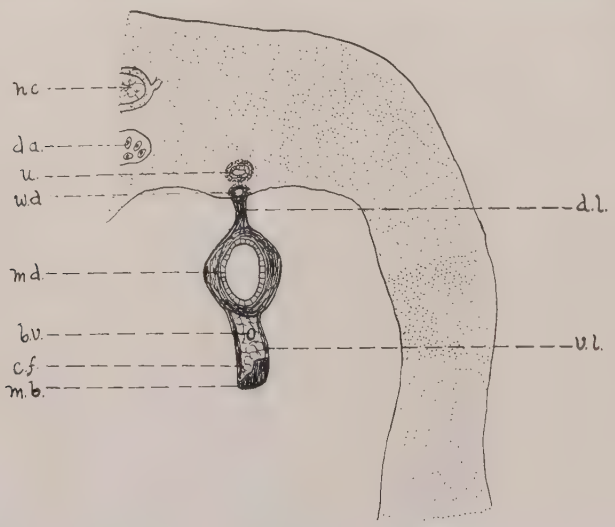

Fig. 6 Transverse section through the mullerian duct of a 20-day-old female chick embryo immediately cephalad to the shell gland showing the ligaments and trace of the wolffian duct (× 30).

has completely degenerated and disappeared. Caudad to the ostium region, however, the mullerian duct remains attached to the wolffian body by a mesenchymatous strip which appears as the backward continuation of the similar strip at the ostium region.

Stages III to IV (10 and 11 days old). The constricted middle region of the caudal portion of the urinogenital ridge appears narrower in stage III (fig. 3). In stage IV it appears to be distinctly divided into a ventral and a dorsal portion, and the 2 remain connected by the constricted strip-like portion

(fig. 8). In the cephalic region of the urinogenital ridge, however, no marked change is noted.

Stage V (12 days old). In the caudal region the strip-like portion connecting the ventral with the dorsal portion of the urinogenital ridge appears narrower than at the previous stage. This strip becomes vascularized at this stage. The remaining portion of the wolffian duct is in a stage of rapid degeneration and its epithelial lining is invaded by mesenchyme cells. The strip of mesenchyme which connected the caudal portion of the urinogenital ridge to the diverticulum-like projection of the dorsal body wall in the previous stages, ceases to exist at this stage and consequently the former remains directly attached to the latter (fig. 9). The major portion of the wolffian body (corresponding to the entire sexual and the major part of the non-sexual division of the urinogenital ridge) has degenerated and the mullerian duct remains attached to the dorsal body wall (fig. 11).

Stage VI (15 days old). The dorsal region of the caudal portion of the urinogenital ridge has merged with the adjoining body wall. The diverticulum-like projection of the dorsal body wall observed in the previous stages appears more or less triangular at this stage and the mullerian duct remains attached to the apex of this projection by the constricted strip-like portion of the urinogenital ridge (fig. 12). The ureter is situated inside this triangular projection. Portions of the wolffian duct have completely degenerated but traces of the duct, however, are still visible. Only a small rudiment of the wolffian body (corresponding to a rudiment of the non-sexual division of the urinogenital ridge) is left. Throughout its entire course, the mullerian duct remains attached to the dorsal body wall by a continuous sheet of mesenchyme or the dorsal ligament.

Stage VII (17 days old). Muscular tissue of the plain type and blood vessels are observed throughout the dorsal ligament but very few collagenous fibers are seen. Traces of the wolffian duct are left only in isolated locations along the caudal region.

The triangular projection of the dorsal body wall referred to above is no longer seen at this stage. The latter appears flat like the adjacent regions (fig. 4).

Stages VIII and IX (20 and 21 days old). The dorsal ligament extends from the fourth thoracic rib down to the cloacal region. It is made up mostly of muscular tissue with few collagenous fibers interposed. Traces of the wolffian duct can still be seen in the caudal region (fig. 6). In the cephalic region a small rudiment of the wolffian body is left (vide stage VI).

APPENDIX

It is a well established fact that the wolffian duct eventually degenerates in the female chick embryos but the details of this process are not mentioned in the literature. Lillie ('27) suggested that it degenerates along with the wolffian body. From a review of the above it is evident that the process of degeneration of the wolffian duct is closely associated with the embryonic development of the dorsal ligament. The process starts with the degeneration of only the anterior end of the duct on the eighth day (stage I) but afterwards goes on with great rapidity in a caudad direction with the result that it ceases to exist in the entire cephalic portion of the urinogenital ridge by the ninth day (stage II). In the caudal portion of the urinogenital ridge the degeneration of the wolffian duct is slow and irregular, that is, not in a cranio-caudad direction as in the cephalic region. The major portion of the duct in this region, however, totally disappears by the twentieth day but traces are left in some isolated locations even at hatching time. It is interesting to note that the extreme cranial end of wolffian duct degenerates along with the wolffian body as suggested by Lillie but further caudad the former disappears earlier (stage II). During the process of degeneration the epithelial wall of the duct is invaded by cells of mesenchymatous nature (fig. 14), but the actual transformation of 1 kind of cell into the other is not seen. Incidentally, in the male chick embryos similar histological changes take place in the epithelial wall of the mullerian duct which eventually degenerates after the eighth day (Lillie, '27).

B. Morphology and histology of the ligaments in immature chicks

a. The ventral ligament. The ventral ligament is a thin membrane which extends along the mid-ventral region of the

oviduct from the funnel and ends as a thin muscular cord in the caudal end of the shell-gland. It remains attached to the oviduct by its dorsal margin while the ventral margin lies free in the body cavity. A transverse section through the ventral ligament in the magnum region (fig. 15) shows that the muscle fibers are concentrated in the free and in the attached margins of the ligament. The lateral margins, however, are bordered by comparatively thinner muscle fibers which are continued round the outer musculature of the oviduct. The musculature of the lateral margins anastomose with each other by very thin muscular trabeculae. The spaces between trabeculae are rather loosely packed with bundles of collagenous fibers which appear as finely dotted areas separated from one another by broken angular lines. Triangular fibroblast cells are situated in the spaces between the collagenous fibers. The cytoplasm of these cells takes up deep stain and each contains a spherical nucleus.

The caudal end of the ventral ligament, as previously stated, is a muscular cord and does not contain any collagenous fibers. It finally breaks up into very thin bundles of muscle fibers which extend down to the utero-vaginal junction (fig. 7, a to c).

b. The dorsal ligament. The dorsal ligament remains attached to the oviduct by its ventral margin while the dorsal margin is attached to the body wall. It extends from the caudal end of the oviduct up to the fourth thoracic rib. The bulk of the ligament is made up of muscle fibers with very little connective tissue interposed. A transverse section through the dorsal ligament in the magnum region shows that the musculature of the lateral margins are continued round the outer musculature of the oviduct. Near the attached end a small area of the ligament is filled with collagenous fibers (fig. 16).

C. Growth and differentiation of the ligaments

Like the oviduct its ligaments also exhibit 2 distinct growth phases in the post-hatching period. The first covers a considerable period of time (up to about the 20th week of age)

during which the ligaments exhibit slow but steady growth (Isogony). This period is followed by one of extremely rapid growth (Heterogony) (vide table 1).

In this period of sudden growth the ligaments become highly vascularized and their musculature also becomes very thick. The ventral ligament is seen to be closely packed with bundles of collagenous fibers. It might be mentioned that this period of heterogonic growth does not alter the relation of the ligaments which exists at hatching time, that is, the ventral ligament extends from the posterior end of the funnel down to the

TABLE 1

Summary of the measurement of the oviduct and the ligaments in the post-hatching period.

AGE IN WEEKS	NUMBER OF BIRDS	AVERAGE LENGTH OF THE OVIDUCT IN CM	AVERAGE LENGTH IN CM		AVERAGE DIAMETER IN CM (IN THE MAGNUM REGION)	
			Ventral ligament	Dorsal ligament	Ventral ligament	Dorsal ligament
6	5	8	7	10	0.8	1
10	6	8.3	7.2	10.5	1	1.3
13	4	9	8	11	1.5	1.8
15	5	10	9	11.5	1.6	2
18	5	10.5	9.5	12	2	2.5
20	4	11	10	13	2.2	2.8
21	5	25	20	30	4.5	5.2

caudal end of the uterus and the dorsal ligament from the caudal end of the oviduct up to the fourth thoracic rib. It is interesting to note that the growth phases similar to those of the ligaments and the oviduct are exhibited by the comb and wattles (Greenwood, '35). For a considerable period of time in the post-hatching period they show isogonic growth, then, with the onset of sexual maturity (after about the 20th week), follows a period of heterogonic or sudden growth.

D. Ligaments in mature chicks

a. The ventral ligament. The ventral ligament is a fan-shaped structure which remains attached to the oviduct by

its dorsal margin while the ventral margin lies freely in the body cavity. It measures about 6 cm in diameter in the magnum region. At the cranial end the ligament remains attached to the posterior elongation of the funnel. Caudad to the funnel the free ventral margin of the ligament is a muscular cord from which bundles of fibers radiate on either side dorsally and become continuous with the outer muscle layer of the oviduct (Curtis, '10). These muscular bundles are thicker than those of immature chicks and frequently anastomose with

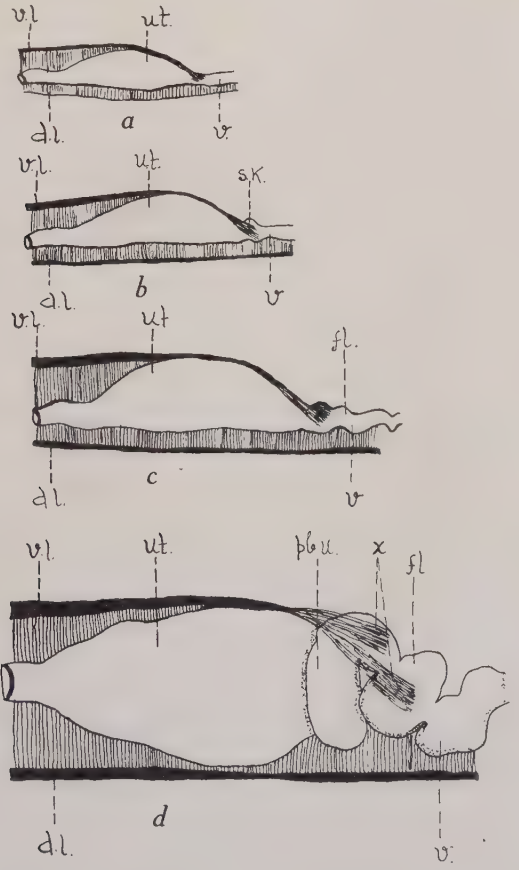

Fig. 7 Diagrammatic representation of the utero-vaginal region of the oviduct and the ligaments in chicks of varying ages. For further explanation see the text.

each other. The irregular-shaped compartments formed by these anastomosing muscular bundles are closely packed with numerous bundles of collagenous fibers.

The caudal portion of the ventral ligament terminates as a solid muscular cord, without any collagenous bundles, over the posterior blind sac of the uterus. This solid cord breaks up into bundles of fibers which pass on to the ventral and ventro-lateral sides of the convoluted anterior portion of the vagina (fig. 7, d x). These muscular bundles are very conspicuous in a mature bird and have been referred to as the utero-vaginal musculature (Greenwood and Blyth, '38).

b. The dorsal ligament. The dorsal ligament maintains a line of attachment to the dorsal body wall from the caudal end of the oviduct up to the fourth thoracic rib. It measures about 7 cm in diameter in the magnum region. The ventral margin of the ligament, however, remains attached to the oviduct. Only the cranial portion of the dorsal ligament is spread out in the form of a "Fan" (Curtis, '10). In the utero-vaginal region it is more or less straight. The dorsal ligament is made up mostly of muscular tissue with very little connective tissue interposed. The muscular bundles of the ligament originate from the mid-dorsal margin and pass ventrally and finally continue round the outer muscle layer of the oviduct (Curtis, '10).

E. Function of the ligaments

It has been mentioned in the previous section that the caudal end of the ventral ligament terminates as a solid muscular cord over the posterior blind sac of the uterus and finally breaks up into thick bundles of fibers. The latter radiate on to the ventral and ventro-lateral sides of the peculiar "S" anterior portion of the vagina. As in the other regions of the oviduct thick bundles of muscle fibers from the dorsal ligament continue round the utero-vaginal region. The muscular bundles from the cord-like end of the ventral ligament, together with those of the dorsal ligament, are responsible for the maintenance of the vaginal convolutions. In fact, the latter

can be straightened out only after the removal of the musculature of the ligaments. Greenwood and Blyth ('38) observed that a number of experimental pullets derived from eggs injected with oestrin at an early stage of incubation, laid shell-less eggs. On post-mortem examination of the oviduct it was noticed that the peculiar "S" convolutions were absent at the anterior end of the vagina and the latter appeared practically straight. On the basis of this observation they suggested that "The function of the peculiarly convoluted anterior portion of the vagina is to prevent the premature passage of the egg through the shell-gland before it has received its calcareous deposit. The absence of adequate vaginal flexures allowed of no resistance to the passage of the eggs through these regions normally characterized by well-developed musculature, the action of which determines the movement of the egg, and were therefore conducted without pause through shell gland and vagina to the exterior.[2]

Examination of the vaginal portion of chicks of varying age reveals certain peculiarities which suggest that a close relationship exists between the development of the vaginal convolutions and the growth and differentiation of the ligaments. In chicks up to the tenth week of age the vagina appears as a straight tube which runs from the caudal end of the uterus down to the cloaca (fig. 7, a). In chicks 11 to 13 weeks old, a slight kinking is observed in the anterior portion of the vagina

[2] It might be mentioned in this connection that during routine post-mortem examination several cases were encountered where the shell-gland contained an egg which was, however, without any calcareous deposits. Obviously the birds were killed immediately after the migration of the egg from the isthmus into the shell-gland. An attempt to push the egg from the latter down into the vaginal portion, however, proved futile. Next, the musculature of the ligaments were removed and the convoluted anterior end of the vagina was straightened out. Then by repeated application of gentle pressure at the isthmo-uterine junction it was found possible to bring the egg into the vagina. Incidentally, during laying the pointed end of the egg is normally extruded first but the eggs which project deeply into the posterior blind sac of the uterus may be turned with the blunt end caudad during the act of laying (Olsen and Byerly, '32). It is not unlikely that musculature of the ligaments plays an important role in the turning of eggs, the cause of which, however, is still unknown.

(fig. 7, b). This kinking eventually takes the form of a sharp bend (15 to 20 weeks) (fig. 7, c). Finally, after about the twentieth week, the anterior portion of the vagina exhibits convolutions characteristic of a laying hen (fig. 7, d). It might be recalled here that up to the twentieth week of age the ligaments are rather inconspicuous structures with poorly developed musculature. After this period the ligaments exhibit sudden growth. They become very conspicuous with their highly-developed musculature which in the vaginal region maintains its convolutions.

DISCUSSION

Curtis ('10) suggested that the strip of thickened coelomic epithelium or the tubal ridge which gives rise to the mullerian duct is also responsible for the formation of the ventral ligament. However, it has been shown in a previous paper (Kar, '47) that the rubal ridge proliferates cells to help in the process of formation of the outer mesenchymatous tunic of the mullerian duct. This process of cell proliferation is completed by the eighth day, as a result of which the tubal ridge ceases to exist and its thickened epithelium appears flat and similar to the adjacent peritoneal epithelium. The present investigation, therefore, does not substantiate Curtis' suggestion.

Curtis ('10) observed that "During the first four or five months after hatching the growth of the oviduct and its ligaments is about proportional to the growth of the rest of the body. With the approach of functional activity (egg laying) the isthmus, albumen portion and funnel of the oviduct elongate considerably. This elongation includes the enclosing peritoneum," Kaupp ('18) also expressed a similar view. The present studies are in full accord with those of Curtis ('10) and Kaupp ('18). However, these authors have not described the histology of the ligaments in immature chicks and their differentiation with the approach of sexual maturity. These points have been dealt with in the present studies.

Curtis ('10) and Hewitt ('39) have made no mention of the relationship between the convoluted anterior end of the vagina

and the ligaments. It has been suggested here that the musculature of the ligaments is responsible for the maintenance of the vaginal convolutions. The latter, according to Greenwood and Blyth ('38) help to keep the egg in the shell-gland for adequate calcareous deposit.

Within recent years the hormonic activities of the ovary have been satisfactorily established (vide Allen, '39). It is maintained that the head furnishings and the oviduct in the fowl are under the hormonic control of the ovary (Pezard, '27; Domm, '31; Greenwood and Blyth, '30 and '32; Greenwood, '35; Juhn et al., '32; Champey, '31). Since the ligaments are intimately connected with the oviduct and show the same growth phases it is not unlikely that they are under hormonal control. However, this statement should be regarded as tentative, subject to revision on the acquisition of experimental data.

SUMMARY

1. The embryonic development of the ligaments have been studied.

2. The morphology and histology of the ligaments in immature chicks have been described.

3. The growth phases of the ligaments and also their differentiation with the approach of sexual maturity have been dealt with.

4. It is suggested that the ligaments are under the hormonal control of the ovary.

5. It has been suggested that the ligaments are responsible for maintaining vaginal convolutions.

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PLATE

ABBREVIATIONS

b.v., blood vessel.
c., constriction.
c.f., collagenous fibers.
d.a., dorsal aorta.
d.b., dorsal body wall.
d.v., diverticulum-like projection of the dorsal body wall.
d.l., dorsal ligament.
fl., flexure.
m.d., mullerian duct.
m.b., blocks of muscular tissue.
m.s., mesenchymatous strip.
m.t., muscle strands.
m., muscular tissue.
mc., mesenchymatous cells.
n.c., notochord.
o.d., oviduct.
pb.u., posterior blind sac of the uterus.
s.k., slight kinking.
u., ureter.
ut., uterus.
v.l., ventral ligament.
v., vagina.
w.d., wolffian duct.

PLATE 1

EXPLANATION OF FIGURES

8 Photomicrograph of the transverse section through the caudal portion of the urinogenital ridge of an 11-day-old female chick embryo. (× 30.) For further explanation see text.

9 Photomicrograph of the transverse section through the caudal portion of the urinogenital ridge of a 12-day-old female chick embryo showing the ventral and the dorsal ligaments (× 30).

10 Photomicrograph of the sagittal section through the cephalic region of the mullerian duct of an 8-day-old female chick embryo showing the mesenchymatous strip (× 30).

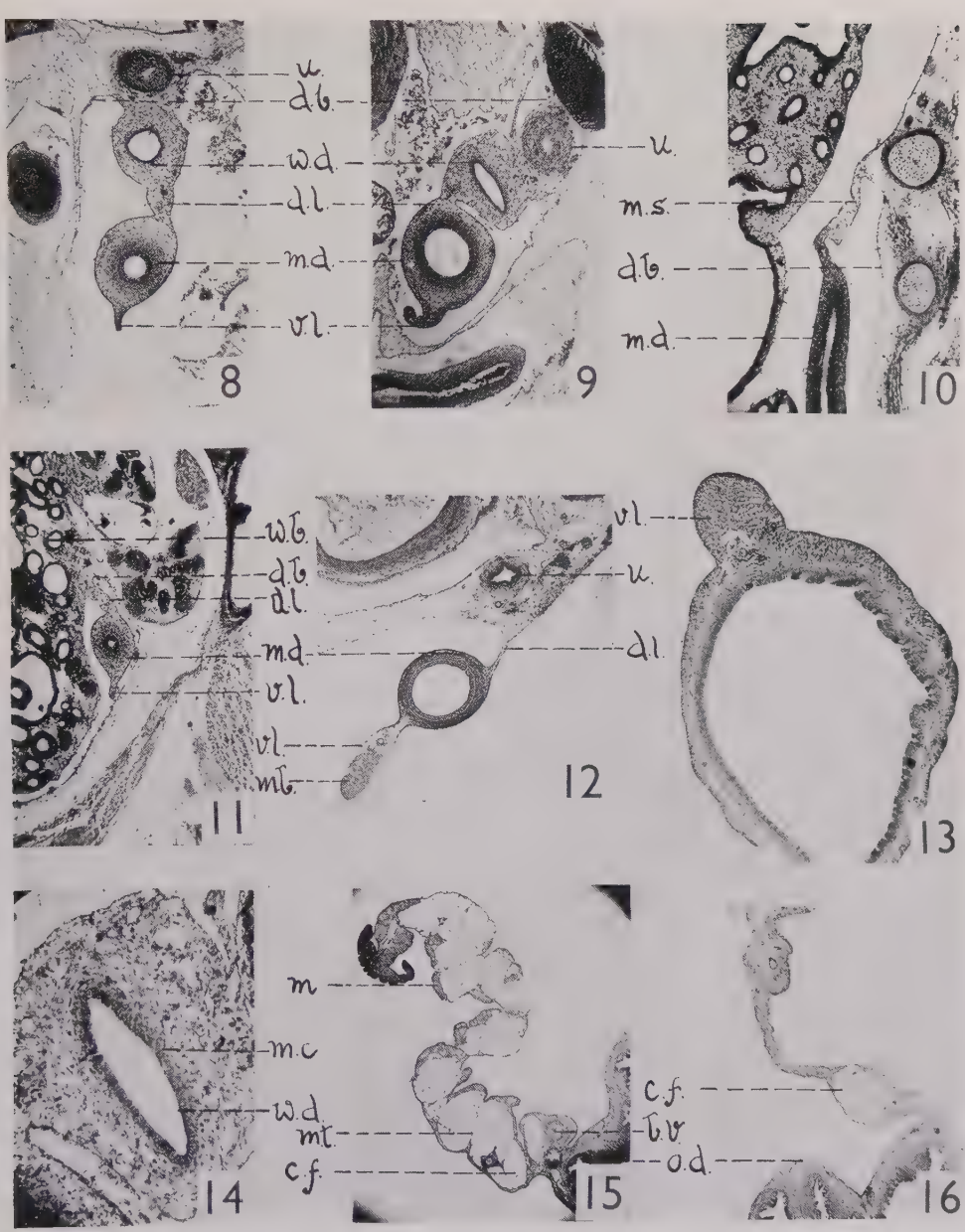
11 Photomicrograph of the transverse section through the cephalic region of the mullerian duct of a 12-day-old female chick embryo showing the dorsal ligament (× 30).

12 Photomicrograph of the transverse section through the mullerian duct of a 15-day-old female chick embryo showing the ligaments (× 30).

13 Photomicrograph of the transverse section through the shell-gland of a 20-day-old female chick embryo showing the ventral ligament (× 35).

14 Photomicrograph of the transverse section through the caudal portion of the urinogenital ridge of a 12-day-old female chick embryo showing the wolffian duct (× 80).

15 and 16 Photomicrographs of the transverse sections through the ventral and dorsal ligaments respectively of an 80-day-old female chick (× 20).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THE COSTOMEDIASTINAL BORDER OF THE LEFT PLEURA IN THE PRECORDIAL AREA

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TWO FIGURES

INTRODUCTION

It has long been apparent that a considerable discrepancy exists between the traditional textbook treatment of the margin of the left pleura in the precordial area and the usual findings in dissection of the region. This investigation was made to determine the magnitude of this discrepancy and to establish if possible the normal topographic relations of this margin with the sternum and costal cartilages.

The problem exhibits many facets, among which may be mentioned the level at which the left pleura emerges from the sternal margin, its mean distance from the sternal margin at each subsequent costal or intercostal level, its corresponding extreme positions and their variations, and the character of the curve (concave or convex to the median plane) of the left pleural margin.

Review of the literature

Bourgery, writing in 1835, stated that Heister's observation that the inferior portion of the border of the left pleura leaves the sternum and lies behind the costal cartilages was the basis for the practice of penetration of the pericardium by an aspirating needle along the left lower border of the sternum. Bourgery noted from his personal observations, however, that the pleura frequently remains close to the

sternal margin and recommended caution in following Heister's suggestion.

Luschka's data (1863) largely established the traditional teaching concerning the relations of the left pleural margin. He stated that although variability exists, in most cases he found, in the adult, distances from the sternal border to the left pleural margin of 1.5 cm at the level of the fifth costal cartilage, of 2 cm at the level of the sixth cartilage, and 3.5 cm at the seventh. He did comment, however, that the left pleura is frequently so little divergent that the intermediate space is reduced to a minimum.

During the following years a number of studies appeared which largely refuted or modified the conclusions of Luschka. Luschka, himself, noted the findings of Nuhn (1860) who concluded that there is normally insufficient space to reach the pericardium without perforating the pleura. Sick (1885) analyzed the condition in 23 adult cadavers and found the left pleura at the fifth cartilage under or just at the border of the sternum in 18 cases, in a comparable position at the level of the sixth cartilage in 10 cases, and just leaving the sternum at the seventh cartilage in 8 cases. In his 12 cases of children the pleural margin showed a still closer adherence to the sternal border. Tanja (1891) criticized Luschka's description as being based on impressions rather than statistical analysis. Tanja found no asymmetry of the pleural borders in lower mammals or monkeys and more divergence, but no essential asymmetry in higher apes. His 42 human cases comprised 28 foetal, newborn and infant bodies, and 14 between the ages of 8 and 76 years. Tanja emphasized the considerable variability in the anterior margins of both pleural sacs and the variation in level at which the left margin leaves the sternum (third rib to ensiform cartilage). His tracings of pleural margins tend to invalidate the concept of a cardiac notch in the pleura, i.e., a deviation similar to but less extensive than that of the left lung. Ruge ('10) found that an outbowing of the pleural margin due to the heart appeared to be rare among monkeys, the right and left lines coming

down straight and parallel behind the sternum in the usual case. The apes show a wider separation of the 2 margins but both behave similarly, there being no cardiac notch. In man he reported that the left pleural margin begins to deviate opposite the sternal end of cartilages 6, 5, 4 or even 3. He stated that it has a gentle curve with a left sided convexity. Among his tracings of the pleural borders in 12 human cases, only 1 shows a marked cardiac notch and only 2 others show any left-sided convexity of the left margin.

Similarly Brooks (1889) found the left pleural margin entirely under cover of the sternum in 4 of 7 cases and quite close to the sternal border in 1 other cadaver. He concluded that the area of pericardium uncovered by pleura was normally very small.

Noback ('32) made a brief note pertinent to this question in relation to his observations of 100 adult cadavers. He observed that the left pleural margin does not usually deviate at the level of the fourth costal cartilage, but that, on the contrary, in 90 cases the right and left pleural reflections were in close contact behind the sternum and extended thus inferiorly to the sixth or seventh costosternal junction. He concluded that the customarily described area of pericardium devoid of pleural covering in this region is not present.

Merkel (1899 and '02) stated of the left pleural border that the usual curved course of the line is altered in the region of the pericardium by the fact that the right directed convexity is changed to a concavity and that the sixth rib is not reached at its sternal insertion, but somewhat farther out along its cartilage. His figure has been copied extensively and shows the left pleura diverging abruptly from the right at the level of the fourth rib insertion, crossing the fourth interspace slightly lateral to the sternal margin, being further separated from it in the fifth interspace and following the lower border of the sixth cartilage laterally. The figure of Poirier (1898) shows a more gradual curve of divergence of the left pleural border, the convexity of which reverses itself only slightly. It shows the pleural margin at the sternal border in the

fourth interspace but well away from it in the fifth and sixth intervals. He described a slight internal concavity which his figure only just indicates. This figure more nearly approaches my own findings (fig. 2) than any other in the literature, although showing greater divergence to the left than the mean position of the present study.

Rauber-Kopsch ('07) described the left pleural margin as exhibiting an easy left-sided outbowing which does not quite reach the cardiac notch of the left lung. His figure, which is also copied in 1 current anatomical text, shows a rather extreme divergence from the sternal border at the level of the fourth interspace with wide intervals between the pleural margin and the sternum in the fifth and sixth interspaces.

Further discussion of figures and descriptions in texts and reference books would be fruitless. Suffice it to say that Merkel's figure is basic to illustrations in Gegenbaur (1890) and Corning ('07) and is similar to that of Testut (1894 and later editions). That of Rouvière ('24) is modified from Merkel and shows no clear interval in the fourth interspace but is otherwise the same. Merkel's figure also appears with occasional modifications in the currently standard anatomical textbooks (Gray, Morris, Cunningham). Such figures show a reversed curve in the left pleural margin, its lower portion having a left-sided convexity as it withdraws from the sternal margin through the levels of the fifth and sixth costal cartilages. Descriptive matter in these texts reflects, however, the realization of recent revising editors that the Luschka tradition is in error, in that they place the left pleural margin near (at or beyond) the sternal border at the levels of the fifth and sixth ribs and interspaces. Piersol has taken cognizance of the more serious investigations of the pleural margins. He describes the left margin as passing from behind the sternum at about the junction with the sixth cartilage and denies the traditional teaching that the left pleura takes a curve similar to that of the left lung (cardiac notch). His figure, however, shows a left-sided convexity in the lower substernal part of the margin.

Radiological studies have been useful in determining the inferior pleural margins and the pleural relations in the superior mediastinal area, but have contributed little on the contour of the pleural borders in the precordial area.

AUTHOR'S MATERIALS AND METHODS

One hundred and two consecutive adult cadavers were used in this investigation. Following removal of the skin, fascia and pectoral musculature in routine class dissection, headless pins were inserted through the sternal extremities of the fourth, fifth and six intercostal spaces. These passed immediately against the sternal margin and by means of a probe were pushed vertically downward through the intercostal membrane and muscles into the tissues of the mediastinum. The sternoclavicular joint having been disarticulated, the upper 6 ribs were cut at their angles and removed together with the manubrium and body of the sternum. This exposed the pleural sacs, the pericardium and the great vessels, and the pins protruding from these structures marked accurately the sternal extremities of the fourth, fifth and sixth intercostal spaces. The margin of the left pleura was then defined by gently freeing it from the mediastinal areolar tissue. Measurements (accurate to 0.5 mm) were then made of the distance (either to right or left) between the pleural margin and the pins marking the left sternal border. A free-hand sketch was made of the relationship of the pleural margin to the pins and in a considerable number of instances map pins with large heads were substituted after the measurements were taken and a permanent record was made photographically (fig. 1). A long straight-edge was also employed to mark the midline of the body and measurements of the pleural margin were made to it in the fourth, fifth and sixth interspaces.

Effect of disease

Of the 102 specimens examined 7 were discarded because of extreme displacements due to disease, leaving 95 cases for

consideration. Pulmonary lesions are frequent in cadavers and in general produced no significant shift of pleural borders. Large hearts, likewise, produced as a group no noticeable deviation of the pleural margins. Such conditions appeared to produce no greater deviation of the pleural borders than was exhibited in the variations among the "normal" group. Thus, there are included in the data all except the most extreme cases of gross lesions. The effect of this is to widen the range but should have little effect on the mean position of the pleural margins.

ANALYSIS OF DATA

A. Level of deviation of left pleural margin

The 2 pleural margins usually lie closely approximated behind the upper part of the body of the sternum slightly to the left of the midline. In this study the left margin began to deviate from the right anywhere from the third rib to the fifth interspace. At times this divergence is so gradual as to be equivocal and consequently the data here reported covers 80 cases. The mean level of deviation is that of the fourth rib. 70% of the 80 cases showed divergence of the left margin at some level between the third and fourth interspaces.

B. Level of emergence of pleura from left sternal margin

This point varied in the 86 cases giving reliable data from the third interspace to the seventh costal cartilage, the mean level being the upper third of the fifth intercostal space. Emergence of the pleura at levels from the fourth interspace to the lower border of the sixth costal cartilage, inclusive, accounted for 73% of all cases.

C. Relations of pleural margin at the fourth intercostal space

Ninety-five cases gave information on this relationship. Measurements were made both to the left sternal margin and to the midline of the body.

Mean position — 0.6 cm to the right of the left sternal margin, i.e., substernal in position or 1.16 cm from the midline.

Total range — 1.5 cm to left of (lateral to) the sternal margin to 3 cm to its right or 3 cm to the left of the midline to 0.7 cm to its right. In 76 cases the border was substernal and in 19 cases to the left of the sternal margin.

Range of 70% of cases grouped around the mean. 1 cm on either side of the mean includes 70% of the cases (slightly over 1 standard deviation).

D. Relations of pleural margin at the fifth intercostal space in 95 cases

Mean position — 0.2 cm to the left of (lateral to) the left sternal margin or 1.9 cm to the left of the midline.

Total range — 2.5 cm left of the sternal margin to 2.5 cm to its right or from 0.1 to 4.4 cm to the left of the midline.

In 27 cases the border lay substernally, in 5 cases at the sternal margin, and in 63 cases to the left of it.

Range of 70% of cases grouped around the mean — 0.8 cm on either side of the mean related to the sternal margin or 1 cm on either side of the mean related to the midline are the limits of this group.

E. Relations of pleural margin at the sixth intercostal space in 94 cases

Mean position — 1.1 cm to the left of sternal margin or 2.5 cm to left of midline.

Total range — 1.5 cm to right of sternal margin to 3.5 cm to its left or 0.5 to 5.7 cm to the left of the midline. In 10 cases the pleural border lay substernally, in 3 at the sternal margin, and in 81 was to its left.

Range of 70% of cases grouped around the mean. 1 cm on either side of the mean includes this group.

Horizontal measurements taken to the left of the sternal margin at the sixth interspace rapidly reach points under the sixth costal cartilage; thus the mean position of the pleura

as determined in this study locates the pleural margin under the sixth cartilage. The mean pleural margin crosses the sixth interspace at a distance of 1.5 cm from the sternal border as measured along the sloping under border of the sixth costal cartilage, measurement being made on a life-size figure (fig. 2). A distance of 2.5 cm from the sternal margin similarly measured marks the limit in that direction of the central 70% of cases.

F. Curvature of the left pleural margin in 94 cases

An analysis of photographs and tracings of the left pleural margin with respect to its curvature may be summarized as follows:

Marked convexity to right	7 cases
Slight convexity to right	22 cases
Essentially straight-line contour	30 cases
Slight convexity to left	24 cases
Marked convexity to left	11 cases

More than 80% of the cases fall into the central classifications of essentially uncurved or slightly convex to right or to left.

G. Validity of the data

Internal evidence of the data gives some indication of its validity. When the mean positions of the pleural margin are located on a life-size sketch of the anterior chest wall incorporating the outline of an average sternum, the measurement of the mean from the left sternal margin and from the midline check within 0.5 mm of each other at all 3 interspace positions. Further, all points lie on a smoothly defined curve, which intersects the sternal margin at the level determined by the data (fig. 2).

DISCUSSION AND SUMMARY

It is unfortunate that no method has been devised as yet to visualize the lower costomediastinal border of the left pleura in the living. Radiographic studies have elucidated

its upper portion (Stephani and Kirsch, '33, and others) and the inferior pleural border (Lachman, '42) but the radiologic density of the heart is at present a barrier to use of this method in the precordial area. However, cadaver study has been and is of considerable value.

The early studies of this pleural segment (Luschka, 1863) emphasized an extreme position perhaps due to the examination of highly pathological material and were perhaps influenced by the lack of proper fixing and hardening agents.

The comparative investigations of Tanja (1891) and Ruge ('10) are of interest in showing no differences between the right and left margins of the pleura in lower mammals or monkeys and no essential asymmetry in their relations among the apes. The studies of these authors and of Sick (1885) with respect to the relations in man were perhaps based on too few observations but indicated clearly that Luschka's findings did not represent a true mean. Tanja stressed the variability of the pleural position in the costomediastinal region, a point of view which this study fully corroborates. Its variability emphasizes the necessity of examining a large number of specimens.

To recapitulate the main results of this study, the left costomediastinal pleural border has the following mean relationships. It diverges from its fellow of the right side at the level of the fourth cartilage, lies substernally 0.6 cm medial to the left sternal margin at the level of the fourth intercostal space, emerges from the cover of the sternum in the upper third of the fifth intercostal space and moves only 0.2 cm lateral to the sternum in the midregion of this interspace. Measured from the sternal end of the sixth interspace it lies 1.1 cm lateral to the sternal margin, which measurement is a location under the sixth costal cartilage due to the considerable slope of this cartilage. The curve defined by these points passes the lower border of the sixth costal cartilage 1.5 cm from the sternal end of that interspace measured along the sloping lower border of the cartilage.

Examination of the central grouping of the data reveals that plus or minus 1 cm from the mean at all levels measured includes 70% or more of the cases. This proportion of cases is cited as being comparable to 1 standard deviation in statistical treatment. The mathematical computations of a true standard deviation were not undertaken.

The character of the curvature of the left pleural border as traced and photographed is instructive with respect to traditional illustrations. There was a marked convexity to the left in only 11 cases and to the right in only 7 cases. In 76 of the 94 cases the border was relatively straight or showed a slight curvature (see fig. 1). A slight convexity to the left was scarcely more frequent (24 cases) than to the right (22 cases).

The variability of the pleural margin is considerable. The total range of location of the various points for all cases is 5 cm at the fifth and sixth interspaces and slightly less at the fourth. It is possible that somewhat less variability occurs among essentially normal living individuals.

Comparison of figure 2 with illustrations in current texts and reference works emphasizes their differences. The pleural margin in the precordial area in the latter will be found to adhere closely to the more laterally placed of the line of crosses of figure 2. Since this line marks 1 limit of the central group of 70% of my cases, then approximately 85% of pleural margins lie more medialward than the average line of heretofore acceptable illustrations.

This study has emphasized the variations occurring in a cadaver group in the lower costomediastinal border of the left pleura and has established the mean relationships and central tendency of this margin in a large number of cases. It is hoped that it will provide a broad and at the same time a more precise view of thoracopleural topography in the precordial area.

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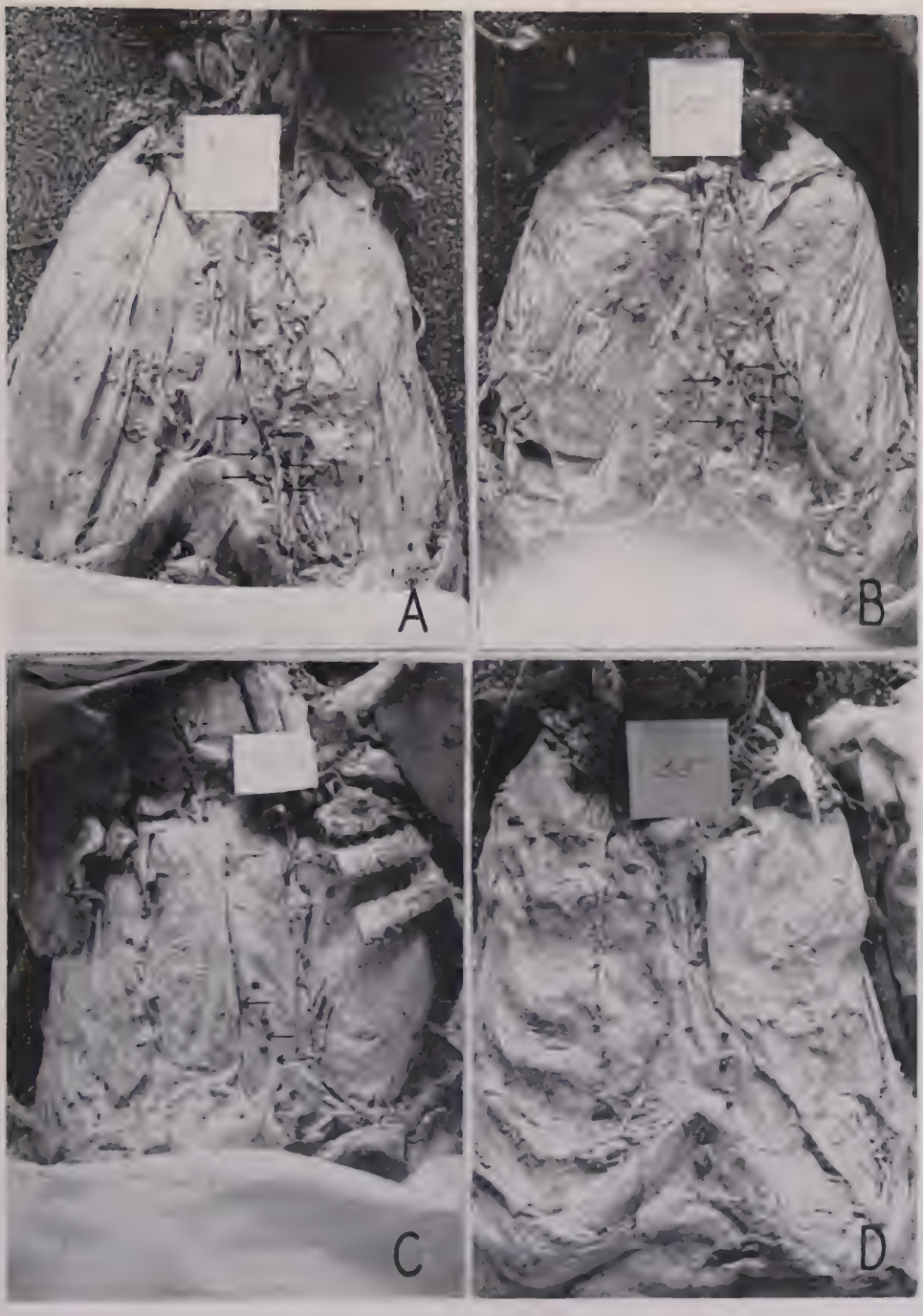
PLATE 1

EXPLANATION OF FIGURE

1 Examples of photographic records of the left pleural reflection. A and B show conditions close to the mean, C a more medially placed and D a more laterally placed pleural margin. The pins mark the sternal extremities of the fourth, fifth and sixth interspaces. Arrows directed to the right call attention to the pins, those directed to the left emphasize the position of the left pleural margin.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B
C
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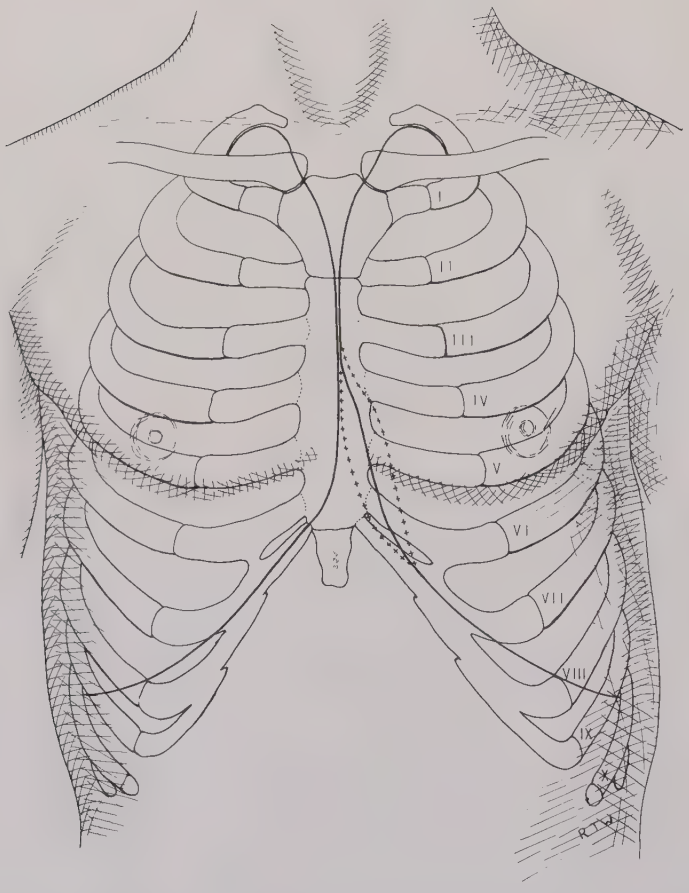

2 The relations of the pleural reflections to the anterior chest wall. The solid line on the left represents the mean. The interrupted lines (crosses) in the precordial area represent variants between which lie 70% of the cases studied.

CHROMAFFIN PATTERNS IN BIRD ADRENALS

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THREE PLATES

The chromaffin and interrenal tissues of the bird adrenal are intermingled to such an extent that they cannot properly be designated medulla and cortex. Indeed, in some species chromaffin tissue may lie on the outer surface of the gland. When there is a relatively small amount of this tissue, it is scattered through the interrenal tissue in the form of islets of various sizes. As the amount increases, the islets are replaced by inter-connecting strands that may become so extensive as to fill the gland with a meshwork.

Upon sectioning, the chromaffin tissue forms various patterns, depending upon the amount of tissue and, to some extent, upon the species. To determine the characteristic chromaffin pattern of a species it is necessary to study the serial sections of a number of individuals in order to rule out unusual arrangements. Sometimes the chromaffin tissue will be shifted to 1 side or tend to be concentrated so that a section through 1 part of the gland will give a much different pattern from that found in another part.

From a study of several species of birds, we have presented some typical examples.

METHODS

All birds were adults.

The adrenals were fixed in formaldehyde and potassium bichromate, and stained with hemalum after sectioning.

Drawings were made with the aid of a camera lucida. In large adrenals, these have been reduced in size in order to facilitate comparison.

OBSERVATIONS

The pelican adrenal is of particular interest since it is the largest in proportion to body weight of any bird known (Hartman). As is usually true for other vertebrates this is due to the greater amount of interrenal tissue. The chromaffin tissue, which is confined to the interior of the gland, occurs as small irregular islets (plate 1). In the cormorant which belongs to the same suborder there is more chromaffin tissue, the islets being larger and many of them elongated.

Only 2 hawks were studied, the Red-shouldered and the Cooper's Hawk (plate 1) of which the latter will serve as an example. Here the large amount of chromaffin tissue forms an almost complete meshwork of attractive pattern. Isolated islets are rare. Both species are somewhat similar.

In the Bob-White (plate 1) there is a pattern of numerous large, elongated, irregular patches partly united. An appreciable amount of chromaffin tissue is found on the surface of the gland.

Six species of woodpeckers (Flicker, Pileated (plate 1), Red-bellied, Yellow-bellied Sapsucker, Hairy and Downy) were studied. The patterns show similarity. Although the woodpeckers possess adrenals which are relatively among the smallest (Hartman), the chromaffin tissue is not unusually large in amount. A characteristic feature of the woodpecker adrenal is the occurrence of numerous large sinuses scattered through the interrenal tissue, which would indicate a rich blood supply. Chromaffin tissue is found in the periphery. In the Downy Woodpecker, which was more thoroughly studied than other species, this tissue constitutes a large proportion of the periphery.

Three species belonging to the Family Paridae, the Black-capped and Carolina Chickadees (plate 1) and the Tufted Titmouse, were studied. All have a relatively large amount

of chromaffin tissue which is distributed as fairly large, often elongated, islets with little anastomosis. Sinuses are frequent in the islets. Chromaffin tissue constitutes much of the surface of the adrenal in some specimens of the Titmouse.

Both the Golden-crowned and Ruby-crowned Kinglets (plate 1), were compared. The relative amount of chromaffin tissue is greater than that in the Paridae.

Adrenals from a few species of the Family Icteridae were examined. They were the Meadowlark, Red-wing and Cowbird. Their chromaffin patterns are quite similar. The figure of the Cowbird adrenal is typical (plate 1).

Considerable study was made of the Family Compsothlypidae. The following species were examined: Black and White Warbler, Nashville Warbler, Parula Warbler, Yellow Warbler, Magnolia Warbler, Black-throated Green Warbler, Myrtle Warbler, Blackburnian Warbler, Chestnut-sided Warbler, Bay-breasted Warbler, Black-poll Warbler, Yellow Palm Warbler, Oven Bird, Northern Waterthrush, Yellowthroat, Canada Warbler and Redstart. Several are shown in plate 2. It will be noted that species from the same genus may show greater difference than species from different genera. Compare *Dendroica aestiva* with *Dendroica virens*. There is a similarity in pattern among different individuals, at least in some species of warblers (Redstart, plate 3).

The following species of the Family Fringillidae were compared: Cardinal, Rose-breasted Grosbeak, Purple Finch, Goldfinch, Red-eyed Towhee, Savannah Sparrow, Slate-colored Junco, Tree Sparrow, Chipping Sparrow, White-throated Sparrow, Lincoln's Sparrow, Swamp Sparrow and Song Sparrow. All have a considerable number of islets in the pattern, with a surprising limitation of connections between the islets for the amount of chromaffin tissue involved. The White-throated and Swamp Sparrows serve as examples (plate 3).

DISCUSSION

From this limited survey it appears that the distribution of chromaffin tissue is at least partly determined by the rela-

tive amount present. If small it occurs as islets throughout the gland with little or none at the periphery. As it increases, the islets become elongated and tend to unite until the amount of material becomes so large that the gland becomes permeated with a meshwork of chromaffin substance. With the increase, more of the chromaffin tissue appears on the periphery so that sometimes it covers a considerable area.

As in other structures of the body chromaffin patterns tend to be characteristic of the species. This is well shown in the Redstart. There may also be resemblances among species of different Families, e.g. Pelican and Cormorant. But the same Genus may show considerable difference in the amount of chromaffin material although one may see a suggestion of similar pattern, e.g. Dendroica.

Müller in discussing the adrenals of the fowl says that there is always more medullary (chromaffin) tissue in the interior of the gland than in the periphery. He also found, in general, an increase in the chromaffin cells with increasing age. The observations in the pigeon were similar. In young birds, the chromaffin tissue cells formed only a few cell groups which were connected by means of almost imperceptible bridges. With increasing age the chromaffin tissue increased and showed a strand-like arrangement. In old birds the chromaffin cells might form a more or less distinctly connected peripheral border due to their increased number.

SUMMARY

The pattern of the chromaffin tissue of the bird adrenal depends somewhat on the relative amount of this tissue in the gland. When small it is arranged as distinct islets scattered through the interrenal tissue. As it increases in amount the islets become larger, and often elongated, tending to unite. If a relatively large proportion of chromaffin tissue is present, it may form a meshwork throughout and also cover appreciable amounts of the surface of the gland.

Characteristic patterns may be found in certain species. However, there may be considerable variation in the amount of chromaffin material among species of the same Genus.

We are indebted to Dr. E. H. Behre for the use of the Louisiana State University Laboratory at Grand Isle, La., and to Mr. Richard Archbold for the facilities of the Archbold Biological Station, Lake Placid, Florida. The following gave valuable assistance in the collection of material: Richard Archbold, Leonard J. Brass, Robert Goslin, Dr. Jonathan S. Thatcher, Dr. Wallace McNutt, and Frank Mead.

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PLATE 1

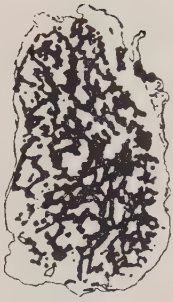
Patterns in some different families of birds.

Pelecanidae, Pelican *(Pelecanus occidentalis carolinensis)*; *Phalacrocoracidae,* Cormorant *(Phalacrocorax auritus)*; *Accipitridae,* Cooper's Hawk *(Accipiter cooperi)*; *Perdicidae,* Florida Bob-White *(Colinus virginianus floridanus)*; *Picidae,* Northern Pileated Woodpecker *(Ceophloeus pileatus abieticola)*; *Paridae,* Carolina chickadee *(Parus carolinensisca rolinensis)*; *Sylviidae,* Eastern Ruby-crowned Kinglet *(Corthylio calendula calendula)*; *Icteridae,* Eastern Cowbird *(Molothrus ater ater)*.

Large adrenals have been reduced and small ones enlarged to approach more nearly the same magnitude for better comparison of patterns.

PELECANUS

PHALACROCORAX



ACCIPITER

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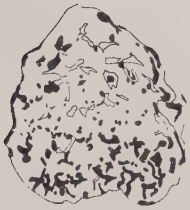
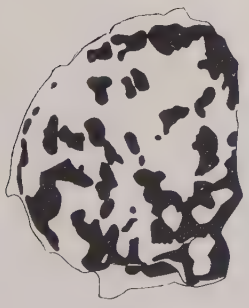


GEOPHLOEUS

PARUS



CORTHYLIO

MOLOTHRUS

PLATE 2

Generic and specific differences in the *Compsothlypidae*.

Black and White Warbler *(Mniotilta varia)*, Nashville Warbler *(Vermivora ruficapilla ruficapilla)*, Northern Parula Warbler *(Compsothlypis americana pusilla)*, Eastern Yellow Warbler *(Dendroica virens virens)* Oven-bird *(Seirus aurocapillus)*, Northern Water-thrush *(Seiurus noveboracensis noveboracensis)*, Northern Yellow-throat *(Geothlypis trichas brachidactyla)*, Canada Warbler *(Wilsonia canadensis)*.

MNIOTILTA

VERMIVORA

COMPSOTHLYPIS

DENDROICA
AESTIVA

DENDROICA
VIRENS

SEIURUS
AUROCAPILLUS

SEIURUS
NOVEBORACENSIS

GEOTHLYPIS

WILSONIA

PLATE 3

Different individuals of the American Redstart *(Setophaga ruticilla)*. Also examples of Fringillidae; the White-throated Sparrow *(Zonotrichia albicollis)* and the Swamp Sparrow *(Melospiza georgiana)*.

SETOPHAGA RUTICILLA

ZONOTRICHIA ALBICOLLIS

MELOSPIZA GEORGIANA

THE MAMMARY GLAND DEVELOPMENT IN MALE MICE AT NINE WEEKS OF AGE[1]

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TWENTY-TWO FIGURES

A study of the structure of the mammary glands of male mice was undertaken to determine their normal appearance in certain strains. This was done in order to detect variations from the normal should they be produced in experimental animals. It was anticipated that the glands would be very small since a number of workers have mentioned their lack of development.

Turner and Gomez ('33) said that the glands of the male mice remain rudimentary during normal adult life. Dalton ('45) referred to the work of Turner and Gomez and made the statement that "there is very little postnatal growth in the mammary gland of the male mouse, and it remains a rudimentary structure, consisting of a short main duct with a few rudimentary branches." A photomicrograph of an adult male strain C mouse was shown to illustrate a normal gland. According to Shimkin ('45) the mammary glands of male mice are stunted. Lacassagne ('36) said that normally in male mice the mammary glands remain completely rudimentary.

The present investigation started with an examination of the A and C57 leaden strains. It was found that the glands of the male A strain mice were small compared to some very well-developed glands of the male leadens. Therefore, it was

[1] This work has been supported in part by grants from The Anna Fuller Fund, The Jane Coffin Childs Memorial Fund for Medical Research and the National Cancer Institute.

decided that a study of several stocks might be of value to determine whether or not there were characteristic differences between them. Nine different lines were examined. All of them were highly inbred except the Swiss. The lines of mice employed for this investigation were the A, c^e, C_3H, C57 black line 6 (B), C57 brown line cd (Br^{cd}), C57 brown line a (Br^a), C57 leaden (Ln), dba line 1 and Swiss (Sw).

The technique used in making total mounts of the mammary glands was somewhat modified from that used at the National Cancer Institute. The skin was slit up the mid-dorsal line and carefully removed from the body wall so that the mammae were left adherent to the removed skin. This was pinned flat with fur side down on a layer of paraffin in a pan and covered with Dubasque Brasie fixative. After 8 to 24 hours of fixation the specimens were placed in 80% alcohol for a few hours. The mammary glands which were prepared as whole mounts were separated from the muscle and connective tissue with the aid of a binocular dissecting microscope. They were placed on a cover slip and by pressing the gland rather firmly with filter paper some of the excess fat was removed. The tissue usually remained adherent to the cover slip during the staining process. The gland was stained with Mayer's hematoxylin, destained in 2% HCl in 70% alcohol and then placed in a saturated solution of lithium carbonate to bring out the blue color. The tissue was carried through the alcohols and xylene and mounted in balsam.

Whole mount slides were made of some of the largest of the third mammae of the males from each of the strains at 9 weeks of age. Similar mounts were made of glands from animals 6 weeks old, 12 weeks old and a few that were older. Mammae of 6 weeks old females were prepared for comparison. In the males there were no glands found which seemed to correspond to the fifth mammary glands of the females. In all of the tables only the 3 thoracic pairs and the fourth pair, located in the abdomino-inguinal region, were considered. The glands are numbered in an anterior to posterior direction.

All of the glands of the 9 weeks old males were measured with calipers and the data were recorded for each group as shown in table 1. In order to condense this information, the

TABLE 1

Size of individual mammary glands in ♂ C57 brown (cd) mice 9 weeks old.

NO. OF MOUSE	SIZE OF MAMMAE IN MM. (LENGTH × WIDTH) I (1st)		II (2nd)		III (3rd)		IV (4th)	
	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.	Lt.	Rt.
1	7 × 4	5 × 3	9 × 5	5 × 3	16 × 10	0	12 × 5	12 × 5
2	0	6 × 4	0	0	7 × 5	0	0	0
3	0	0	5 × 3	0	9 × 7	0	0	0
4	7 × 4	12 × 3	8 × 3	0	8 × 7	0	0	0
5	0	0	5 × 3	8 × 4	10 × 6	8 × 5	0	0
6	Min.	0	4 × 2	Min.	3 × 1	0	0	0
7	0	0	0	0	0	0	0	0
8	10 × 5	9 × 3	15 × 4	10 × 3	16 × 15	10 × 10	0	18 × 5
9	7 × 3	0	0	10 × 4	0	0	0	0
10	0	0	5 × 3	0	0	3 × 1	0	0
11	9 × 4	0	0	7 × 4	0	0	0	9 × 3
12	0	0	6 × 3	9 × 4	0	0	0	0
13	0	0	10 × 3	9 × 3	0	0	8 × 3	0

TABLE 2

Comparison of size groups of mammae at various sites in 13 ♂ C57 browns (cd) 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	61.5	3.8	3.8	19.2	11.5	0	0	0	0
II	34.6	3.8	19.2	26.9	15.4	0	0	0	0
III	61.5	0	7.7	3.8	15.4	0	3.8	3.8	3.8
IV	80.8	0	0	7.7	7.7	3.8	0	0	0

data were summarized in a table for each strain (tables 2 to 11). The "0" in the tables indicates that no gland was found. "Minute" indicates that the gland length times its width was less than 1 mm^2; 1 to 16 indicates that it would fit into an area from 1 mm^2 up to but not including 16 mm^2. Since the

area covered by the third mammary gland is roughly circular or oval in outline, it is easy to visualize the size of the gland. In the upper limits, for instance, those falling in size group 196 to 256 would represent a gland which would fit into an area 14 to 16 mm wide or its equivalent. There is 1 gland (Br^{cd} mouse no. 8) which falls in this group (table 1). This represents 3.8% of the total number of the third mammae.

TABLE 3

Comparison of size groups of mammae at various sites in 29 ♂ C57 leaden 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	62.1	5.2	5.2	17.2	10.4	0	0	0	0
II	27.6	5.2	22.4	25.9	13.8	3.4	0	1.7	0
III	44.8	8.6	12.1	10.4	5.2	10.4	6.9	1.7	0
IV	60.3	0	17.2	5.2	15.5	0	1.7	0	0

TABLE 4

Comparison of size groups of mammae at various sites in 30 ♂ C57 black 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	36.7	18.3	43.3	1.7	0	0	0	0	0
II	28.3	13.1	58.3	0	0	0	0	0	0
III	5.0	5.0	58.3	21.7	8.3	1.7	0	0	0
IV	48.3	8.3	40.0	3.3	0	0	0	0	0

It is interesting to note that about 60% of the total number of the male Br^{cd} mammary glands showed no development.

The lack of bilateral symmetry in the development of these glands was striking. For example, in C57 brown line cd the third mammary glands of the first 4 mice (table 1) were well developed on the left side but none were observed on the right.

There were only 2 animals (nos. 5 and 8) in which the third mammary glands were present on both sides. None of the animals showed development of all the 4 pairs of glands. However, in 2 cases (nos. 1 and 8) it can be seen that all of the glands except 1 were fairly large. Mouse no. 7 showed no development of any of the mammae.

TABLE 5

Comparison of size groups of mammae at various sites in 12 ♂ C57 brown (a) 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	54.2	12.5	33.3	0	0	0	0	0	0
II	62.5	8.3	29.2	0	0	0	0	0	0
III	37.5	8.3	54.2	0	0	0	0	0	0
IV	62.5	4.2	25.0	8.3	0	0	0	0	0

TABLE 6

Comparison of size groups of mammae at various sites in 30 ♂ Swiss 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	20.0	18.3	46.7	13.3	1.7	0	0	0	0
II	16.7	26.7	41.7	13.3	1.7	0	0	0	0
III	20.0	26.7	35.0	10.0	6.7	1.7	0	0	0
IV	36.7	28.3	33.3	1.7	0	0	0	0	0

A similar analysis of the 9 weeks old male mice of the other strains, representing a total of 97 animals, showed the same lack of symmetry in the development of the mammary glands. In general, where there was 1 large gland there were usually others which were also well developed. With the exception of 6 Swiss males there was no case where the first 4 pairs of the mammary glands were all present. On the other hand,

there were only 6 male mice (1 Br^{cd}, 2 c^{e} and 3 C_3H) in which there were no mammary glands found. In 4 strains, Br^{cd}, Ln, B and Swiss, the third mammae were the largest glands observed. The third mammae of the C_3H and the A male mice were the least developed of all the mammary glands. Ninety per cent of the C_3H males had no glands in this region. The few that were present were very small.

TABLE 7

Comparison of size groups of mammae at various sites in 13 ♂ A 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	19.2	3.8	34.6	38.4	3.8	0	0	0	0
II	7.7	3.8	23.1	46.2	19.2	0	0	0	0
III	80.8	15.4	3.8	0	0	0	0	0	0
IV	57.7	3.8	23.1	0	15.4	0	0	0	0

TABLE 8

Comparison of size groups of mammae at various sites in 40 ♂ C3H 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	43.8	2.5	51.3	2.5	0	0	0	0	0
II	33.8	36.3	28.8	1.3	0	0	0	0	0
III	90.0	7.5	2.5	0	0	0	0	0	0
IV	33.8	32.5	32.5	1.3	0	0	0	0	0

As the various tables are analyzed it becomes obvious that strain differences exist in the degree of mammae development and many animals should be examined in order to determine the normal range. Table 11 compares the third mammary glands of the males of the 9 strains and gives an indication of such a range.

So far only the dimensions of these glands have been discussed. Perhaps of greater interest is a study of the structure of some of the largest mammary glands of each strain as illustrated in the photomicrographs. It can be observed that in the third mammary glands of the Ln and Br^{cd} strains that the ducts are wide, show well-defined lumina and that these ducts are branched and possess end-buds (figs. 2 and 13). The

TABLE 9

Comparison of size groups of mammae at various sites in 13 ♂ ce 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	42.3	23.1	30.8	3.8	0	0	0	0	0
II	100	0	0	0	0	0	0	0	0
III	84.6	7.7	7.7	0	0	0	0	0	0
IV	88.5	3.8	7.7	0	0	0	0	0	0

TABLE 10

Comparison of size groups of mammae at various sites in 17 ♂ dba (1) 9 weeks old.

MAM. GL. NO.	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
R & L									
I	85.3	14.7	0	0	0	0	0	0	0
II	67.6	32.4	0	0	0	0	0	0	0
III	61.8	38.2	0	0	0	0	0	0	0
IV	58.8	41.2	0	0	0	0	0	0	0

B and Br^{a} mice that were examined showed narrower ducts and less branching (figs. 8 and 15). An examination of more of the Br^{a} strain would be desirable to determine whether or not any individuals could be found with large glands such as were seen in the Br^{cd} mice.

Table 8 shows that in most of the C_3H mice there was no development of the third mammary gland. The largest one

found was about 1.5 mm long by 1 mm wide (fig. 21). Some of the other mammae were considerably larger. For this reason a photomicrograph of the second mammary gland of a C_3H male is shown (fig. 20). It will be noted that the tubules are very fine and show comparatively few branches. All of the mammae that were present in the dilute browns were minute, consisting of a short main duct with a few rudimentary branches (figs. 16 and 17).

A few photomicrographs of the third mammary glands of females are included to compare the size and structure of

TABLE 11

Size comparison of the third pair of mammary glands at 9 weeks in the males of 9 strains of mice.

TYPE OF MICE	PERCENTAGE OF GLANDS IN EACH SIZE GROUP								
	0	Minute	1 to 16	16 to 36	36 to 64	64 to 100	100 to 144	144 to 196	196 to 256
Br^{cd}	61.5	0	7.7	3.8	15.4	0	3.8	3.8	3.8
Ln	44.8	8.6	12.1	10.4	5.2	10.4	6.9	1.7	
B	5.0	5.0	58.3	21.7	8.3	1.7			
Br^{a}	37.5	8.3	54.2						
Sw	20.0	26.7	35.0	10.0	6.7	1.7			
A	80.8	15.4	3.8						
c^{e}	84.6	7.7	7.7						
C_3H	90.0	7.5	2.5						
dba	58.8	41.2							

these 6 weeks old animals with those of 6 and 9 weeks old males. In a female the duct leading from the nipple is enlarged. In the male both the enlarged duct and the nipple are absent. Gibson ('30) compared the life history of the mammae of 2 strains of albino mice, 1 a low-tumor strain and the other a high-tumor strain. She observed a generally slower glandular development in the mice of the high-tumor strain. Our observations did not include many females, but it is interesting to note that the C_3H and dba animals, which are high tumor strains, showed less glandular development than Ln, Br^{cd} and B females at 6 weeks of age.

DISCUSSION

These data present clear evidence that differences in the development of the mammary glands do exist between the males of diverse lines of mice. This is contrary to the common belief accepted at present by workers in this field as shown by the published data.

A new field is opened up for the investigation of the significance of these differences in their relationship to other physiological states such as carcinoma of the mammary glands. All of the lines of mice studied were closely inbred except for the Swiss. In Heston's review of data on the incidence of mammary gland tumors in female mice ('45) he lists dba, A and C_3H as having a high incidence of carcinoma of the mammary gland, and the C57 black, C57 brown and C57 leaden as having a low tumor incidence. Furthermore, no mammary gland tumors have been recorded in the c^e mice. When a study was made of the mammary glands in the males of these strains we found the reverse of what might be expected. Those mammary glands that were the best developed were found in the males of the low tumor strains while those that showed very little development were typical of males of high tumor strains. Experiments are now under way to investigate the structure of the mammary glands of male hybrids from reciprocal crosses between low and high tumor strains.

Little ('41) and others state that tumors of the breast in mice do not develop unless the animals have the necessary prerequisites that may lead to cancer in this gland. These animals must ordinarily have: (a) the necessary genetic susceptibility, (b) the development of the mammary tree to the necessary stage under the influence of hormones and (c) the mammary tumor inciter or agent such as is found in the milk of high tumor strain female mice.

The existence of strain variations in the mammary gland development in male mice offers a new line of attack on endocrine influences. For example, any influence of the pituitary gland on the development of the mammae may, possibly, be

successfully separated from the effects of ovarian hormones by studying male mice. Similarly the study of a group of castrated males may reveal the interrelationship between the testes and mammary gland development.

The problem of localized developmental differences is also interesting. Deficient development of the mammae in female mice in certain strains shows a high degree of localization according to Little and McDonald ('45). They found that in the C_3H stock 100% of the deficient mammae were at the location for the third gland. This may be correlated with the fact that 90% of the C_3H males studied showed no glands at this same site.

SUMMARY

A comparative study was made of the degree of development of the male mammary glands in 9 strains of mice. These were A, c^e, C_3H, C57 black, C57 brown (lines a and cd), C57 leaden, dba line 1 and Swiss. Skins with all of the mammary glands in position were saved from animals representing different age groups. All of these glands were studied and their comparative sizes tabulated. Whole mounts were made of the third mammary glands from males at 6 and 9 weeks of age. Glands of 6 weeks old females were prepared for comparison. The leaden and brown (line cd) males showed a remarkable extent of development of the mammary glands. Individual glands at 9 weeks sometimes involved an area 1×1 cm or more in extent. Males of other lines showed a lesser degree of mammary gland development in the age groups studied. These findings were contrary to the common belief that the mammary glands of male mice remain rudimentary during normal adult life.

CONCLUSIONS

1. Strain differences exist in the degree of development of the mammary glands of the male mice.

2. In certain of the low tumor strains such as C57 leaden, C57 brown and C57 black the mammary glands of the males may involve an area of 1×1 cm in extent.

3. All these glands do not develop uniformly even within the same animal.

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PLATE 1

EXPLANATION OF FIGURES

All figures are photomicrographs of whole mount preparations of mammary glands.

1 Right third mammary gland of a 6 weeks old C57 leaden male (× 4).

2 Left third mammary gland of a 9 weeks old C57 leaden male (× 4). The area marked off in india ink indicates the field that is shown enlarged as figure 3.

3 Photomicrograph of the area outlined in figure 2. It shows the thin walled ducts and well formed lumena typical in C57 leaden males (× 50).

4 Left third mammary gland of a 12 weeks old C57 leaden male (× 4).

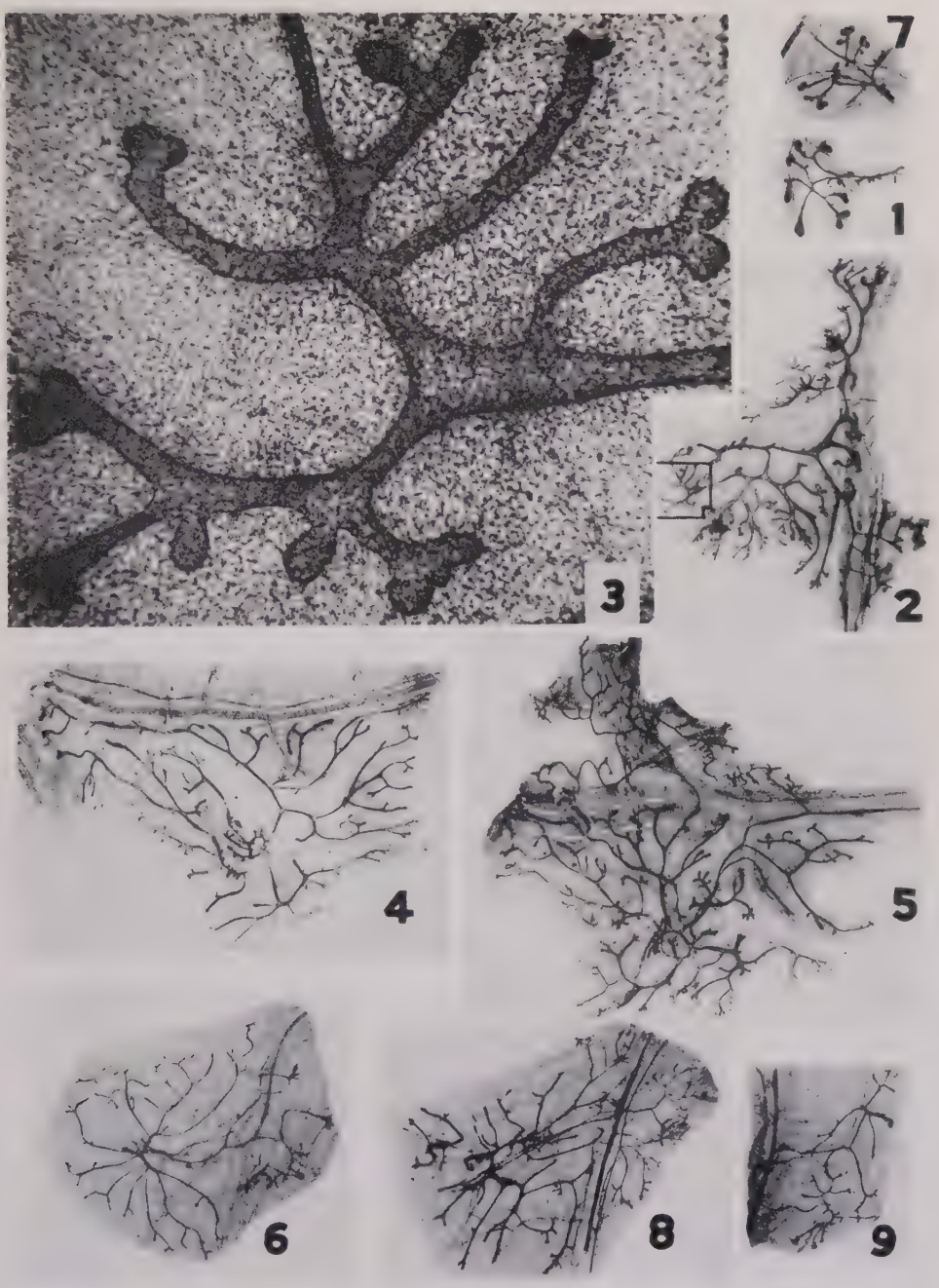
5 Left third mammary gland of a 14 weeks old C57 leaden male (× 4).

6 Left third mammary gland of a 9 weeks old Swiss male (× 4).

7 Right third mammary gland of a 6 weeks old C57 black male (× 4). Note the resemblance to leaden male gland in figure 1.

8 Left third mammary gland of a 9 weeks old C57 black male (× 4).

9 Left third mammary gland of a 9 weeks old A male (× 4).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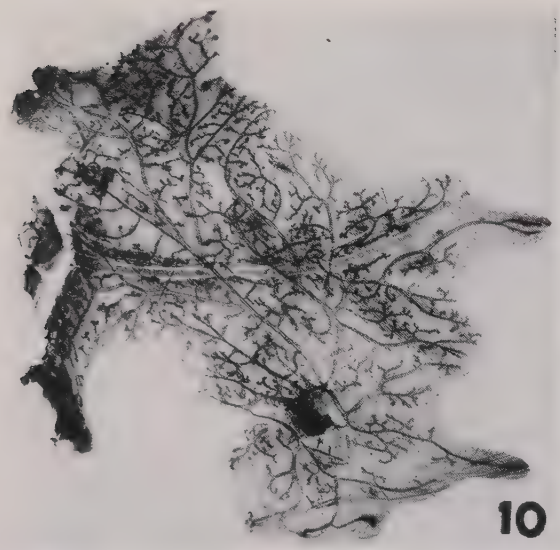
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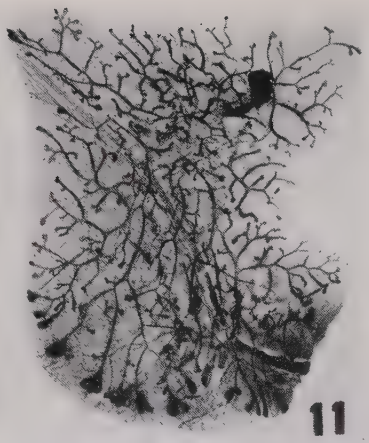
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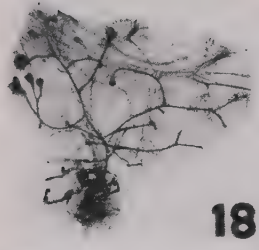
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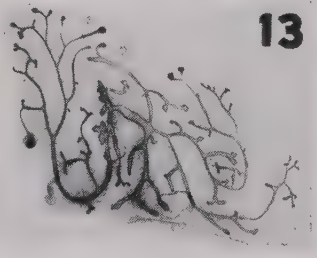
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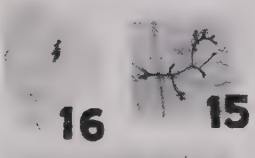
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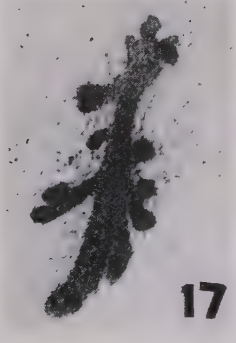
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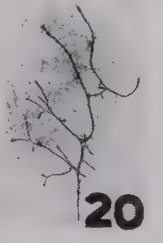
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PLATE 2

EXPLANATION OF FIGURES

All figures are photomicrographs of whole mount preparations of mammary glands.

10 Right third mammary gland of a 6 weeks old C57 leaden female (× 4).

11 Right third mammary gland of a 6 weeks old C57 black female (× 4).

12 Right third mammary gland of a 6 weeks old C57 brown line cd male (× 4).

13 Left third mammary gland of a 9 weeks old C57 brown line cd male (× 4).

14 Left third mammary gland of a 6 weeks old C57 brown line cd female (× 4).

15 Left third mammary gland of a 9 weeks old C57 brown line a male (× 4).

16 Right third mammary gland of a 9 weeks old dba male (× 4).

17 The same mammary gland (see fig. 16) has been enlarged to demonstrate its rudimentary condition (× 50).

18 Left third mammary gland of a 6 weeks old dba female (× 4).

19 Right third mammary gland of a 9 weeks old c^e male (× 4).

20 Left second mammary gland of a 9 weeks old C_3H male (× 4).

21 Left third mammary gland of a 9 weeks old C_3H male (× 4).

22 Left third mammary gland of a 6 weeks old C_3H female (× 4).

THE ACCESSORY REPRODUCTIVE GLANDS OF PARASCALOPS WITH NOTES ON HOMOLOGIES

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ELEVEN FIGURES

INTRODUCTION

The comparative anatomy of the male accessory reproductive glands needs clarification in many groups of mammals. This is especially true in the insectivores where considerable doubt exists as to the exact homologies of the various accessory glands present in the different genera. As often pointed out in the literature on these glands their homologies can only be determined definitely through embryological evidence, a type of evidence available to date on the accessory glands of very few mammals or groups of mammals. In most studies the accessory glands have been classified on the basis of their gross anatomy, relations of their ducts, and microscopic structure in lieu of ontogenetic data.

An attempt is made here to provide ontogenetic data for a genus of American moles, Parascalops, in order to furnish a starting point for clarification of the condition in other genera of American insectivores.

Engle ('26) summarized in tabular form the literature on the accessory reproductive glands of mammals omitting, however, the extensive observations of Kaudern ('07, '10) on insectivores and lemurs. More recently Mossman, et al. ('32) described these glands in the Sciuridae and established criteria for the determination of the homologies of the accessory glands based on a classification proposed by Rauther ('04).

It is these criteria that are followed in proposing homologies for the accessory reproductive glands of Parascalops breweri.

The accessory genital glands of male insectivores have been described by a number of authors in varying degrees of detail. Among the more useful of these papers were those of Rauther ('04), Disselhorst ('04), Grosz ('05), Arnbäck-Christie-Linde ('07), and Kaudern ('07, '10).

MATERIAL AND METHODS

The material on which these observations were based was collected over the past 10 years in New Hampshire and New York and consisted of adult males in breeding and post-breeding condition, immature males, new-born males, and gravid females with fetuses in several stages of development. All material preserved for study was fixed in Bouin's solution and stored in alcohol. Reproductive tracts and fetuses were serially sectioned by the paraffin method. Some were stained in toto with borax-carmine while others were stained with Harris' hematoxylin and eosin.

OBSERVATIONS

Morphology of the male reproductive system

Before presenting data on the classification of the accessory glands in Parascalops a description of the male reproductive system is essential. Its superficial appearance was indicated in a figure by Kaudern ('07), who described the position of the testes, and was briefly described by Eadie ('39). A detailed description is presented here (fig. 1), but no attempt is made to present complete histological data. The nomenclature used in designating the accessory glands is that proposed as a result of this study.

The testes are abdominal in position and lie on either side near the base of the bladder in post-breeding males. They never occupy a scrotum although they move caudad into a posterior extension of the abdominal cavity in the breeding season. Their size ranges from a maximum of 12×7 mm

(longitudinal and cross diameters) in full breeding condition in March to a minimum of 3×2 mm in the fall and early winter. The caput epididymis has the usual relations to the testis but the cauda epididymis is a large sack-like structure lying posterior to and distinct from the testis. At the height of the breeding season it is approximately half as long as the testis but in post-breeding males may equal or exceed it in size. Serial sections show that this structure consists of a well formed sack with a heavy fibrous envelope (fig. 4). The extensive convolutions of the ductus epididymis enter the sack and occupy the postero-medial half; the antero-lateral half is occupied by an extensive convolution of what appears to be the proximal end of the vas deferens, the walls of which are invested with a robust layer of involuntary muscle. This apparently constitutes a special sperm-reservoir. A similar structure was described in Talpa by Disselhorst ('04) who considered it a definitely developed "Samen-resevoir." The vasa deferentia proceed in the usual manner to the dorsal wall of the membranous urethra where they enter the urethra independently on either side of the crista urethralis after running a short distance posteriorly within the urethral wall.

The prostate gland (glandula prostatica) is a large, compact, pear-shaped organ lying ventral to the bladder. In the breeding condition it is relatively huge and may measure 25 mm in length. Although appearing as a single structure it is actually double, the two parts being intimately bound together (fig. 5). The stalk of the prostate attaches ventrally to the membranous urethra near the base of the bladder. Serial sections reveal a separate duct for each half of the gland. These ducts enter the ventral urethral wall and proceed caudally for a short distance in it before entering the urethral canal independently and ventrally by a pair of openings which lie slightly anterior and almost opposite to those of the vasa deferentia (fig. 6).

Cowper's glands (glandulae bulbo-urethrales) are well-developed, paired, oval bodies located on either side of the

union of the crura of the penis. In the breeding season each gland lies just medial to the tail of the epididymis and exceeds it in size measuring, in greatest diameters, 8×4 mm. The ducts of Cowper's glands proceed antero-medially to the dorsal wall of the membranous urethra and penetrate the outer sheath slightly anterior to the mouths of the vasa deferentia and prostate ducts. They then run posteriorly in the outer sheath (fig. 6) before penetrating the urethral wall completely to empty dorsally into the urethral canal near the point of union of the crura penis. The openings of the ducts are very close together but apparently independent in the adults studied (fig. 8). In one newborn specimen they joined in a very short common duct before entering the urethral canal.

The flaccid penis extends caudad and ventrad with an S-shaped flexure to the prominent papilla formed by the prepuce anterior to the anal papilla. The surface of the glans is smooth and unadorned with the rugosities or spines found on the glans in some insectovores (Weber, '27, '28; Pearson, '44). A small os penis is present in it (fig. 9).

A pair of undescribed perineal glands are present in Parascalops. These are prominent structures of relatively large size situated interior to the skin muscle on either side of the genital papilla. They extend cephalad and lie ventral and lateral to the corpus of the penis and Cowper's glands. In adult males they measured 8 mm long and 4 mm wide. These perineal glands are composite structures each consisting of a distinct holocrine and merocrine portion, the former lying dorsal and lateral to the latter (fig. 2). Both portions are compound tubo-alveolar glands enveloped in a common fibrous envelope. There are 4 main ducts to each perineal gland, 2 draining each of the morphologically different portions. These have a definite arrangement in all specimens studied and follow a parallel course caudad and ventrad to the rectal papilla. Each of the 2 ducts from the holocrine half of the gland unites with 1 of the ducts from the merocrine portion (fig. 3) to form 2 short common ducts before opening externally near the extreme anterior edge of the rectal papilla.

Thus the products of the 2 gland types are mixed before emerging on the skin surface and there are 2 openings for each perineal gland on the rectal papilla. The terminal part of each duct of the holocrine portion of the gland is associated with a large hair shaft which extends through the common duct to the skin surface.

Classification and analysis of the accessory reproductive glands of Parascalops

I. Glands arising from the lower part of the ductus deferens (mesonephric duct):

a. Glandulae ampullarum. Absent in Parascalops.

b. Vesiculae seminales. Absent in Parascalops. Gross dissections of adults and serial sections of adults, newborn specimens and fetuses show no trace of the above 2 types of accessory glands.

II. Arising from the endodermal part of the urogenital sinus (prostatic, muscular (membranous), and penile urethra):

a. Glandulae para-urethrales (Littré). Urethral glands of this type were not found in serial sections although crypt-like evaginations of the lining of the membranous urethra occur in adults. These did not appear to be glandular in the specimens sectioned.

b. Glandulae prostaticae. The gross anatomy and duct relations of these glands in the adult were described above. Serial sections of 20 mm fetuses show these glands developing as ventro-lateral evaginations of the urethral epithelium approximately opposite the connection of the vasa deferentia to the urethra in the region of the future crista urethralis (fig. 10). In serial sections of newborn specimens the prostatic evaginations have developed into a pair of coiled tubular structures. These evaginations may be considered as homologous to the ventral lobe of the prostate which develops in the human fetus although becoming insignificant in most human adults according to Lowsley's ('12) observations. Sections of the adult gland reveal a compound tubo-alveolar structure. The

lumina of the canals and alveoli are quite irregular because of numerous foldings and diverticulae. There is no trace of striated muscle in the investing sheath although non-striated muscle occurs.

c. Glandulae bulbo-urethrales (Cowperi). The gross anatomy and duct relations in the adult were described above. In the 20-mm fetus Cowper's glands appear as a pair of solid evaginations from the dorsal wall of the urethral epithelium posterior to the mouths of the vasa deferentia and the prostatic anlagen (fig. 11). At this stage the evaginations have grown dorso-laterally and their distal portions lie at the sides of the intestine. In newborn specimens Cowper's glands appear as well organized branched-tubular glands occupying their definitive positions with duct relations essentially as in the adult. Sections of the adult organ show a compact, compound tubular gland (fig. 7) with a layer of striated muscle in the investing sheath. The ontogenetic origin, anatomy and duct relations of these glands provide sufficient data to consider them as homologous to the bulbo-urethral glands of the human and other mammals for which such data are known.

d. Glandula bulbi (glandula bulbo-urethralis inferior). According to Mossman et al. ('32) this occurs only in the Sciuridae as far as known. It does not occur in Parascalops.

III. Arising from the proximal ends of the Müllerian ducts:

a. Utriculus prostaticus (uterus masculinus or vagina masculina). No trace of this structure was found in adults or newborn specimens. In the 20-mm fetus the fused proximal ends of the Müllerian ducts are present joining the urethra slightly posterior to the attachments of the vasa deferentia (fig. 10) and pass forward between them as a median tubular structure which gradually loses its lumen and terminates as a solid structure after a course of less than 1 mm.

IV. Arising from the ectoderm of the preputial or inguinal region:

a. Glandulae preputiales. No large organized glands of this type are present in Parascalops but sections of the pre-

putial region in adults show very small, simple alveolar glands sparsely and irregularly distributed over the inner surface of the prepuce (fig. 9).

b. Glandulae inguinales. Included in this general category are glands of a sebaceous or sudoriferous nature opening in the inguinal or the perineal region. A pair of perineal glands were described above. Their anlagen were seen as invaginations of the ectoderm of the perineal region in the 24-mm fetus and it is obvious that the adult glands are each an elaboration of 2 sebaceous glands, associated with hair follicles, and 2 sudoriferous glands. Rauther ('04) has described similar glands in Talpa which differ in that the terminal ducts do not join before opening on the skin surface. He termed them rectal glands but since they do not open into the rectum, but externally at the posterior border of the perineum it seems preferable to call them perineal glands and classify them here. They occur in both sexes.

Anal glands are not included in this classification of the accessory genital glands. It may be noted, however, that a definite concentration of tubular glands of a sudoriferous type surrounds the terminal portion of the rectum and that these open individually into the ectodermal lining of the anal canal in Parascalops.

DISCUSSION

Ontogenetic data on the accessory genital glands of insectivores other than Parascalops are not yet available and it will not be possible to make accurate comparisons of homologies in this group until this information exists. The adult morphology and duct relations of the accessory glands of Talpa europea are well known (Rauther, '04; Disselhorst, '04; Grosz, '05; and Kaudern, '10). In addition Kaudern ('10) has stated that there is an essential similarity in the chief accessory glands of Talpa, Mogera, Scalopus, Parascalops, and Myogale. Since this similarity exists it may be conjectured that the accessory glands possessed in common by these talpids are actually homologous and that additional ontogenetic data, when obtained, will affirm this.

With present information it is useless to attempt extensive comparisons with other families of insectivores although Kaudern ('10) has discussed possible homologies among the insectivores on the basis of adult morphology and duct relations. On this basis he believes the prostate of soricids may be homologous to that of talpids although recent workers have called this organ a seminal vesicle on the basis of a superficial resemblance to the seminal vesicles of small rodents (Bramble, '35; Hamilton, '40; and Pearson, '44). The solution to this and similar problems can be found only by further ontogenetic studies.

SUMMARY

The morphology and duct relations of the male reproductive system in Parascalops breweri are described with particular reference to the accessory reproductive glands.

Prostate, Cowper's, perineal, and simple preputial glands are found in this talpid. A sperm reservoir is present in the cauda epididymis and an os penis in the glans.

The accessory genital glands are classified as far as possible on an ontogenetic basis with ontogenetic evidence in support of these views.

The homologies of these glands in talpids are briefly discussed on the basis of available evidence. The difficulties of further comparisons among insectivores generally due to lack of ontogenetic data are noted.

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PLATE 1

EXPLANATION OF FIGURES

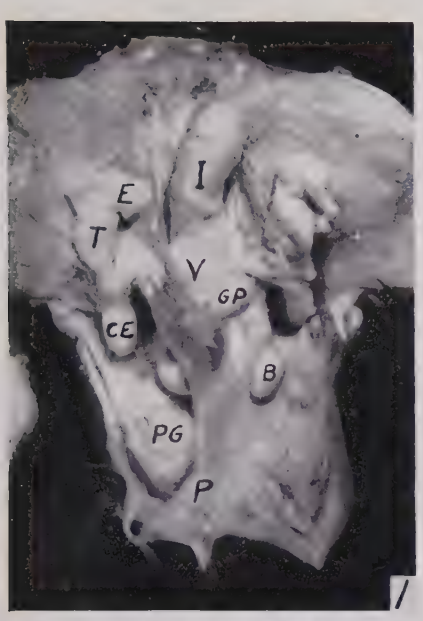
1 Reproductive organs and accessory glands of post-breeding male Parascalops in ventral view. The skin ventral to the penis has been cut in the mid-line and spread laterally to expose the perineal glands. × 2. I., intestine; V,, vesica urinaria; G. P., glandula prostatica; B., glandula bulbo-urethralis; E., caput epididymis; T., testis; C. E., cauda epididymis and sperm reservoir; P. G., perineal gland; P., penis.

2 Section through perineal gland showing holocrine portion above and right, merocrine portion below and left. The intestine is visible, upper left. × 9.

3 Section through skin anterior to rectal papilla showing arrangement of ducts of the 2 perineal glands. The ducts of the holocrine portions of the glands may be identified by their more circular outline and the sectioned hair shaft within each. The ventral pairs of ducts from the 2 morphologically different portions of the glands may be seen uniting on each side. × 45.

4 Section of cauda epididymis showing sperm reservoir. The tubules of the epididymis lie to the right; the large tubules of the sperm reservoir, to the left. × 30.

5 Section of stalk of prostate gland showing paired structure. A portion of the urinary bladder appears above. × 30.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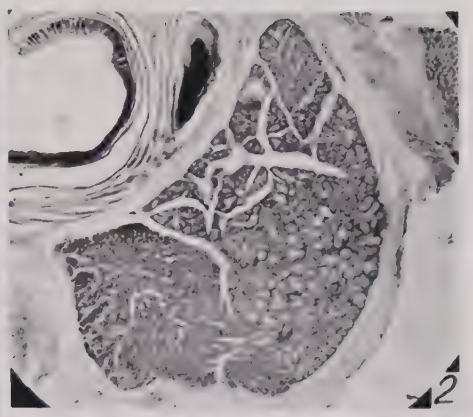
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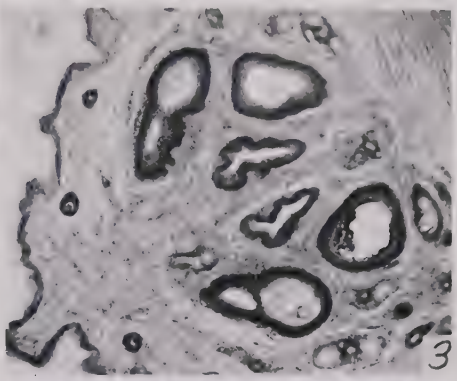
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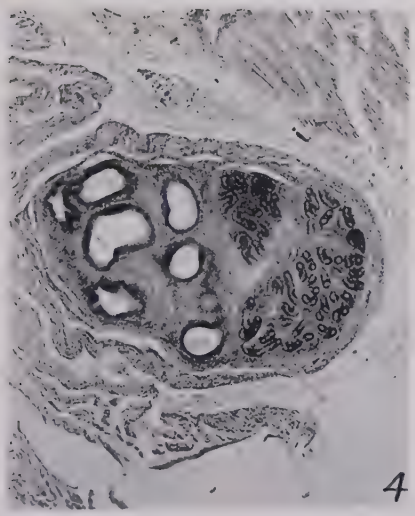
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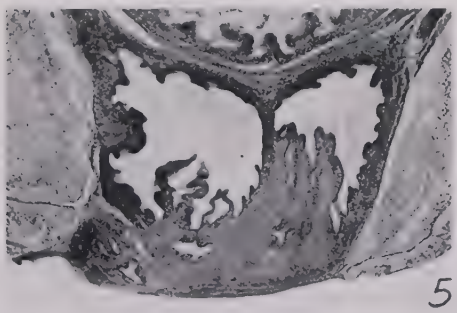
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PLATE 2

EXPLANATION OF FIGURES

6 Cross section through urethra at point of entrance of prostate ducts. The left duct may be seen entering below; the right duct may be seen in urethral wall opposite. The vasa deferentia appear in dorsal urethral wall with the ducts of Cowper's glands lying above and lateral to them. × 40.

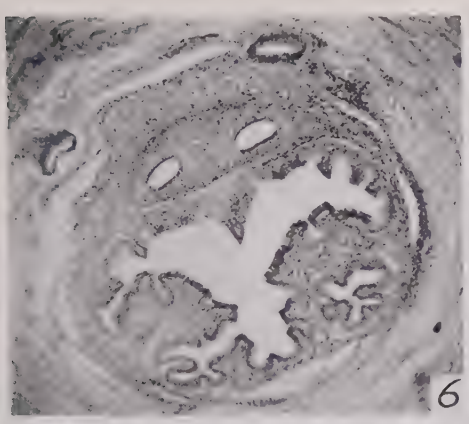
7 Cross section through Cowper's gland in post-breeding male. The intestine appears at left; a portion of the perineal gland, at right. × 15.

8 Cross section through urethra at level of union of crura penis to show entrance points of ducts of Cowper's glands (above). × 40.

9 Section through prepuce and glans of penis. An os penis in a cartilaginous condition appears ventrally in the glans below the urethra. One or 2 small preputial glands project from preputial lining above. × 40.

10 Prostatic anlagen as seen in cross section of urethra in a 20-mm fetus. They appear as a pair of solid ventro-lateral evaginations from the urethral epithelium. The structure appearing opposite (dorso-medially) is the proximal end of the fused Müllerian ducts which disappears in the adult male. The vasa deferentia (not visible) enter the urethra immediately anterior to this point. × 200.

11 Anlagen of Cowper's glands as seen in cross section of urethra in a 20-mm fetus. They appear as a pair of solid, median-dorsal evaginations from the urethral epithelium diverging distally. At this stage their terminal portions (not visible here) lie at the sides of the intestine. × 200.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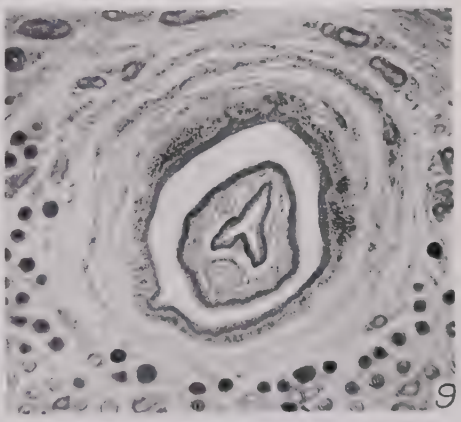
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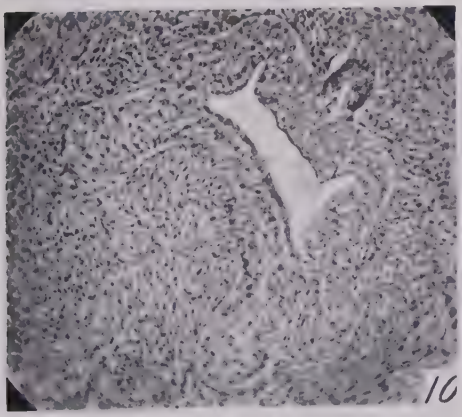
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A STUDY OF THE AMNION WITH THE ELECTRON MICROSCOPE

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ONE FIGURE

INTRODUCTION

While the electron microscope is coming to play an ever-increasing role in biological research because of its tremendous range of practical magnification, yet 2 limitations of the instrument have so far been great handicaps to its application to morphology. These are: first, that the specimen to be examined must be absolutely dry, precluding any study of living or fresh material,[2] and presenting the problem of distortion due to drying; and second, that the specimen must be extremely thin, under 5 micra and preferably 0.3–0.1 micron in thickness. The greater the density of the material, the thinner the specimen must be. This factor of thickness has caused the majority of the difficulties.

Several methods have been used in the preparation of specimens of the required thinness. These are:

a. Wedge-shaped sections can be cut on an ordinary microtome, and the thin portion of the wedge examined. This was the method employed by Richards and Anderson ('42) who found that it was replete with technical obstacles.

b. Claude and Fullam ('45) centrifuged separated cells into groups, which were then placed on a screen and examined.

[1] This work was supported by a grant from the Wisconsin Alumni Research Foundation.

[2] A closed cell for electron microscopy has been described which may make possible the examination of non-desiccated tissue preparations, although, so far as we know, this has not yet been accomplished. (See I. M. Abrams and J. W. McBain, J. Applied Physics, *15:* 607–609.)

c. Cell membranes grown in tissue culture have been examined by Porter, Claude, and Fullam ('45). This method yielded beautiful results, but is limited by what one can grow in tissue culture.

d. A highspeed microtome was introduced by O'Brien and McKinley ('43) and the work with it was ably reviewed by Gessler and Fullam ('46). This method of tissue preparation appears to hold the most promise.

e. The use of extremely thin naturally occurring animal membranes for direct study. This is the subject of this paper.

TECHNIQUE

Living mouse fetuses (circa half-term) were removed from the mother with the membranes intact, and the amniotic cavity injected with 10% formalin by inserting the needle through the placenta. This procedure both stretched the membranes (necessary in order to thin them out), and fixed them in the stretched state. After this they were immersed in formalin for about 24 hours. The amnion was then removed, washed with distilled water, and bits placed on the microscope screens. The specimens were then dried on the screens in vacuo over $CaCl_2$. They were examined at a magnification of 6000.

OBSERVATIONS

a. The nuclei were too thick for adequate study, but in the best preparations their outlines and the 2 or 3 nucleoli which they contain could be seen. These nucleoli must be considerably more dense than the surrounding nucleoplasm as they cast a much darker shadow on the fluorescent screen. The presence of the nucleoli has been checked by staining some of the preparations after electron microscopy and examining with a light microscope.

b. The cytoplasm of the dried membranes appears as a complex spongework of roughly 3 grades of coarseness.

1. A framework of heavy fibers several microns in thickness separated by irregular spaces 5 to 10 μ across.

2. Ramifying between these an intermediate group of fibers averaging about 0.2 μ in thickness which subdivides the larger irregular spaces into rounded intermediate sized ones 1 to 3 μ in diameter.

Fig. 1 Electronmicrograph of internuclear protoplasm of the amnion of a mouse fetus. Formalin fixation. Unstained. The protoplasmic spongework is clearly shown. Small, irregular, dark, poorly defined spots of approximately .2 μ in diameter are of common occurrence in these preparations. Their significance is unknown.

3. The third or finest grade of strands ranges from about 0.1 down to 0.02 μ in thickness and separates rounded vacuole-like spaces about 0.1 to 0.25 μ in diameter.

It is obvious from figure 1 that these 3 gradations in size of protoplasmic strands are quite arbitrary. The strands of all 3 form a continuous whole, a spongework in a very literal sense. Some of the nuclei appear to have an irregular cyto-

plasmic capsule made up of the coarser material from which the coarser strands radiate.

The dried membrane was often seen in the active process of tearing under the stress of the electron beam. The strands have considerable elasticity. When they finally break they snap back like broken rubber bands.

DISCUSSION AND CONCLUSIONS

The morphology recorded is simple and perhaps is partly artifact due to fixation and desiccation. Artifacts of this nature, however, should not be discounted too greatly, for with adequate study their nature could well provide valuable clues to, and even proof of the actual nature of the living material from which they were formed. In the case of the amnion there may be some correlation between its elastic fibrillar structure observed with the electron microscope and the commonly observed contractile motility of the living amnion. We have, however, never detected motility in an amnion of the gestation age which we used in these experiments.

Finally we should like to point out that a number of naturally occurring membranes might be good subjects for electron microscopy. The following seem to us to be worth experimenting with: (1) Unilaminar and bilaminar blastocyst walls, especially of such animals as rabbits, shrews, moles, bats, monkeys, carnivores, and ungulates. (2) Reichert's membrane of rodents. (3) The true chorion of several mammals, such as the carnivores, rodents and ungulates. (4) The various fetal membranes of birds and reptiles. (5) Mesothelial layers which could be peeled from the viscera or body wall, or the mesenteric structures themselves in very small animals or fetuses. (6) The tectorial and Reissner's membranes of the inner ear. (7) The walls of inflated alveoli of the lungs of amphibians and reptiles. Doubtless there are others.

We feel that our observations show the feasibility of electron microscopy of naturally occurring animal membranes. With refinements in technic and more thorough observations

a great deal might be learned about finer cytological structure, for instance of mitotic processes in embryonic membranes, and of the architecture of such structures as Reissner's membrane.

We should like to express our gratitude to Mr. Wallace Mackie of the Soils Department of this University for operating the microscope and giving us other valuable assistance.

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MINIMAL VASCULAR INJECTION OF THE SPLEEN

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ONE FIGURE

A good vascular injection is always expected to be as complete as possible, which means that all vessels should be filled by the injected substance. Moreover, the injected vessels should be well dilated but not overextended.

In the following we are going to show that an incomplete vascular injection reveals in the spleen a special vascular system, which could not stand out distinctly, but would disappear in the general pattern, if the injection had been a complete one.

MATERIAL AND METHODS

India ink was injected into the inferior vena cava or into the heart of anesthetized animals. The quantity of India ink varied from 2 to 4 cm^3, according to the size of the animal. Sixteen white rats, 2 young rabbits and 1 guinea pig were used in these experiments. With the exception of 3 rats, the animals died as a consequence of the injection, by intentional overdosage of the anesthetic, or they were strangled immediately after the injection. Two rats were kept alive for 2 and 23 hours respectively after the injection. For control purposes, a few rats were injected with 1% trypan blue instead of India ink, and 1 rat was given repeatedly India ink intraperitoneally. The spleens of 12 other control animals (rats, guinea pigs and rabbits) which had not been injected, were stained for iron. The organs were fixed in formaldehyde solution immediately after death and embedded in paraffin. Sections of

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various thickness were made of the spleen and other organs. In addition some serial sections were cut of the spleen. Several staining methods were used, mainly haematoxylin-eosin, van Gieson, Mallory-Azan, etc.

RESULTS AND DISCUSSION

An unusual picture was obtained in sections of the spleen of those animals which were injected intravenously or intracardially with small amounts of India ink. This organ showed much of the injected ink to be collected in a space in the outer

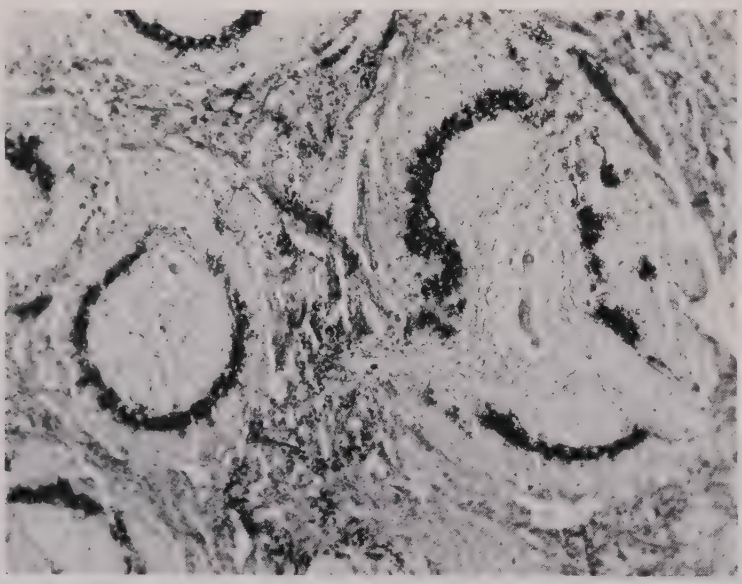

Fig. 1 Rat's spleen. Intravital injection of 2 cm^3 India ink into inferior vena cava. Counterstained with van Gieson. × 60.

region of the Malpighian follicle proper but inside the marginal zone ("border zone" of Andrew) (fig. 1). This space had a smoother inner margin and a somewhat ragged peripheral border. Serial sections revealed that the perifollicular injected space was not a vessel, if one keeps in mind that a vessel has to have a longitudinal axis. The space in question

surrounded the Malpighian follicle and was wanting only in that area where the artery entered the follicle. In the rodent's spleen a fusion of Malpighian follicles is quite frequent and we found therefore a frequent fusion of the perifollicular spaces, which then had an irregular outline conforming to that of the fused Malpighian follicles. Depending on the time elapsed between injection and death, no India ink or only small quantities of it were found in the pulp cords and in the walls of the sinuses.

Though we were unable to find any identical or similar results in the literature, we believe that the spaces filled with India ink were those of the splenic pulp in the marginal zone. The latter appears as dense reticular network in Snook's publication ('44). It seems to be a part of, or at least continuous with, what the author terms lymphatic sheath. A thorough description of the marginal zone is given by MacNeal, Otani and Patterson. According to these authors, it surrounds the splenic follicle or forms its peripheral part. Into it extend and open short capillaries coming from arteries which reach from the follicle into the pulp. They "contain blood excessively rich in formed elements and poor in plasma," since much plasma had passed through the capillary wall inside the follicle proper. These formed elements have to escape through minute openings between the branches of endothelial cells into the pulp spaces. In a later paper, MacNeal described this marginal zone about the follicle as "the most important part . . . as far as action on formed elements in the blood is concerned."

According to the same authors, long capillaries which originate from the same follicular artery, run into the pulp cords where they end. There is always a thin layer of pulp substance between their terminations and the venous sinuses. Such an arrangement suggests that the blood cells passing through them escape into the venous circulation without being subject to much action of the spleen substance. "Due to the slenderness and length of these vessels, they are occluded by passive congestion or by active contraction of the spleen,

by which process relatively more blood is forced into the marginal zone."

Thoma describes a corona of ampullae situated on the border of the Malpighian follicle, originating from arterioles which left the follicle. According to him, there is no open communication between these ampullae and the surrounding splenic pulp.

Robinson investigated the vascular pattern of the spleen. He used injections of "minimal amounts" of substances and also intravital injections of India ink. In the latter case the amount is not mentioned. The author found that "where small amounts are used, the bulk of the gelatine passes through the ellipsoid into the end capillaries and the ampullae and from here scatters into the pulp spaces." No distinction is made between marginal zone and pulp cords.

Andrew recently described a "distinct sinus-like structure between inner and outer zones" of the Malpighian follicle. In our case, we have little doubt that the India ink is retained in spaces of the marginal zone which correspond to Andrew's "sinus-like clefts."

It appears logical to attempt an explanation why so much of the injected ink became piled up on the inside of the marginal zone. MacNeal mentions a slowing up of the current in the pulp spaces of the marginal zone, which favors agglutination and an intimate contact with the pulp cells, permitting phagocytosis of formed elements destined for destruction. But the ultimate factor causing this slowdown of the current and hindering the escape of formed particles is not revealed.

According to MacNeal the capillaries of the marginal zone and the long capillaries of the pulp cords function alternately. In our experiments then, we would have to assume that the long capillaries of the pulp cords were mostly closed. But even then, the sojourn of India ink particles on the inside of the marginal zone is prolonged so much, that we have to accept the view that they are kept back first by the slow-down of the current but later by some other factor, as perhaps ad-

hesion to and partial engulfing by, pulp cells. In the animals who survived for several hours, much of the accumulated ink had again disappeared from the perifollicular spaces, though a small amount was still present there. In the case in which India ink was injected repeatedly intraperitoneally, the spleen showed histiocytes loaded with ink particles to be scattered throughout the entire pulp, and not limited to the perifollicular areas.

Sections of injected spleens and of spleens of 12 control animals were treated after Gömöri for iron. It was found that iron-carrying histiocytes were very numerous in the pulp cords and in the wall of sinuses, but scanty or nearly absent in the follicle proper and in the marginal zone. In the region corresponding to the perifollicular spaces, iron-carrying histiocytes were seen only in 1 control case. There they formed a faint demarcation between the follicle and the marginal zone.

It should be pointed out that the control experiments with trypan blue gave no corresponding results. This may well be explained by the difference in size between the molecules of trypan blue and India ink. We are aware that once the substances are injected, they are no longer to be regarded as simple molecules of trypan blue or as colloidal particles of India ink, but that they become coated with plasma. This has been described by de Haan and by Bennhold. An extensive study of this phenomenon has been made recently by Knisely, Bloch and Warner. Experiments with other substances may lead to a classification as to the molecular size and perhaps the chemical nature of substances, the criterion for the classification being whether they are retained or not in the perifollicular spaces of the marginal zone.

It should not be understood that *all* of the India ink was retained in the perifollicular spaces. As may well be expected many large vessels and some vascular areas contained India ink to a lesser or higher degree. A greater amount of it was usually seen in the capillaries of the lungs, in the glomeruli of the kidneys and in the capillaries of the choroid plexus. Whether this be due to the greater extension of these capillary

networks and hence to a stronger prominence of the injected vessels, to a slow-down of the blood stream, or to both of these factors, could not be decided.

SUMMARY

A small amount of India ink was injected intravenously or intracardially in rodents. It was found that much of the ink had accumulated in perifollicular spaces between the Malpighian corpuscle and the marginal zone. This accumulation of India ink was probably due first to a slowing down of the current in these spaces and followed perhaps by phagocytic action of the lining cells.

Trypan blue injections gave no similar results. This was probably due to the difference in molecular size and chemical nature between the dye and the ink.

In the region of the perifollicular spaces, iron-carrying histiocytes were found only in 1 out of 12 control cases.

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AN UNUSUAL PERITONEAL ANOMALY — A SAC CONTAINING THE JEJUNUM, ILEUM AND TRANSVERSE COLON

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TWO FIGURES

The most common procedure in gross anatomy and at autopsy is the making of an incision through the abdominal wall to expose the organs of the abdominal cavity. Consequently there is astonishment when after this incision has been made the loops of the small intestine are not exposed but are found lying in an additional peritoneal sac. Such sacs have been often described, but they are rarely encountered, and are considered generally as a final condition resulting from a hernia into one of the paraduodenal fossae. The sac to be described differs from those already reported in respect to its contents. Here the sac contained the transverse colon as well as the jejunum and ileum, while in previous cases reported the transverse colon was outside. The abdominal cavity was in every other respect normal, and exhibited nothing which might account for the presence of the sac.

DESCRIPTION OF SPECIMEN

This specimen was found in the Dental Gross Anatomy Laboratory of the University of Pennsylvania in 1944. The subject was a white male of Polish descent, who died of myocarditis at age 73 in Retreat Infirmary, Retreat, Pennsylvania. The patient had been confined to the Infirmary for 3 years prior to his death and neither during this period nor during

earlier life were there any symptoms of digestive or abdominal disease.[1] The incised abdomen revealed a large, thin-walled membranous sac situated in the middle of the abdominal cavity which contained (1) the small intestine, with the exceptions of the first 2 parts of the duodenum and the terminal 3 inches of the ileum, and (2) the transverse colon, with the exceptions of the hepatic and splenic flexures (fig. 2). Along the right side of the sac lay the ascending colon. Superiorly the sac was in contact with the stomach and the right lobe of the liver, while along the left side of the sac lay the descending colon. The greater omentum was normal in appearance and size and was lying in front of the sac. Upon lifting the greater omentum and compressing the stomach cephalad, the superior extent of the sac could be seen. The outer layer of the sac, which judging from its smooth and glistening appearance was obviously peritoneum, was continuous at its basal circumference with the parietal peritoneum of the posterior abdominal wall. A drawing of a mid-sagittal section through the abdomen (fig. 1) displays this continuity of the outer layer of the sac with the parietal peritoneum of the posterior abdominal wall.

The sac was pendulous and bulging due to the size and weight of the loops of intestine contained within it. The wall of the sac was thin enough to permit the outline of the coils of the contained intestine to be recognized. In the post-mortem embalmed state the sac extended into the pelvis but did not completely fill it, so there was an empty space of considerable size. This space was no doubt filled with peritoneal fluid during life and varied in size with the filling and emptying of the intestine. The peritoneal cavity was, however, free from the caseous material which usually remains when ascitic fluid is lost after death. The sac measured about 8 inches in length in its supero-inferior dimension, and approximately 6 inches in the transverse and postero-anterior dimension.

[1] Personal communication from Dr. George T. Baskett, Superintendent Retreat State Hospital, Retreat, Pennsylvania.

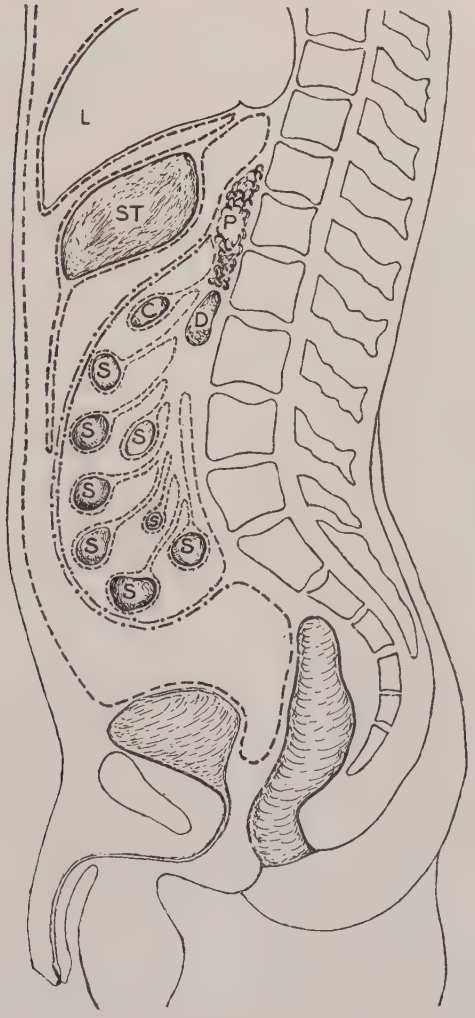

Fig. 1 A drawing showing the sac as seen from the mid-sagittal plane. The normal portion of the peritoneum is represented by a broken line; the sac by a line of dots and dashes.

Abbreviations — L, liver; ST, stomach; P, pancreas; C, transverse colon; D, duodenum; S, ileum and jejunum.

There was a good display of blood vessels in the wall of the sac. They were large and numerous and the arteries were easily followed. The chief arteries were branches of the splenic and superior pancreaticoduodenal arteries. Some smaller arteries were traced to the inferior mesenteric artery. The veins of the sac were traced easily to the lienal and inferior mesenteric veins.

The greater omentum was within the normal range for size. It varied in length from 3 to 8 inches, increasing gradually from the right border to the left. But actually the greater omentum varied greatly from the normal. It consisted only of the anterior lamella of the normal greater omentum — a fold of peritoneum extending from the greater curvature of the stomach as shown in figure 2. The transverse colon and its mesocolon were within the anomalous sac and neither of these structures was in contact with the greater omentum. In this specimen there was therefore no inferior omental recess (fig. 1). The blood vessels supplying the greater omentum consisted of branches of the right and left gastroepiploic arteries, and corresponding veins.

The anomalous sac was incised and its inner surface and contents examined. The inner surface was found to be smooth and glistening and it was obviously peritoneum. It was continuous with the peritoneum of the posterior abdominal wall along an irregularly circular outline. The root of the mesentery was in the normal position. It lay within the sac. Likewise, the transverse mesocolon was in its normal position, and within the sac. As stated previously, the terminal part of the duodenum, the entire jejunum, the ileum with the exception of the terminal 3 inches, and the transverse colon with the exception of the hepatic and splenic flexures, were all in the sac. All of these organs were in their normal positions, as were the other organs of the abdominal and pelvic cavities. All the organs were well preserved and so nearly perfect in shape, size, color and position that the specimen could have served as a model. The jejunum and ileum together measured 11 feet 5 inches in length. The part of the trans-

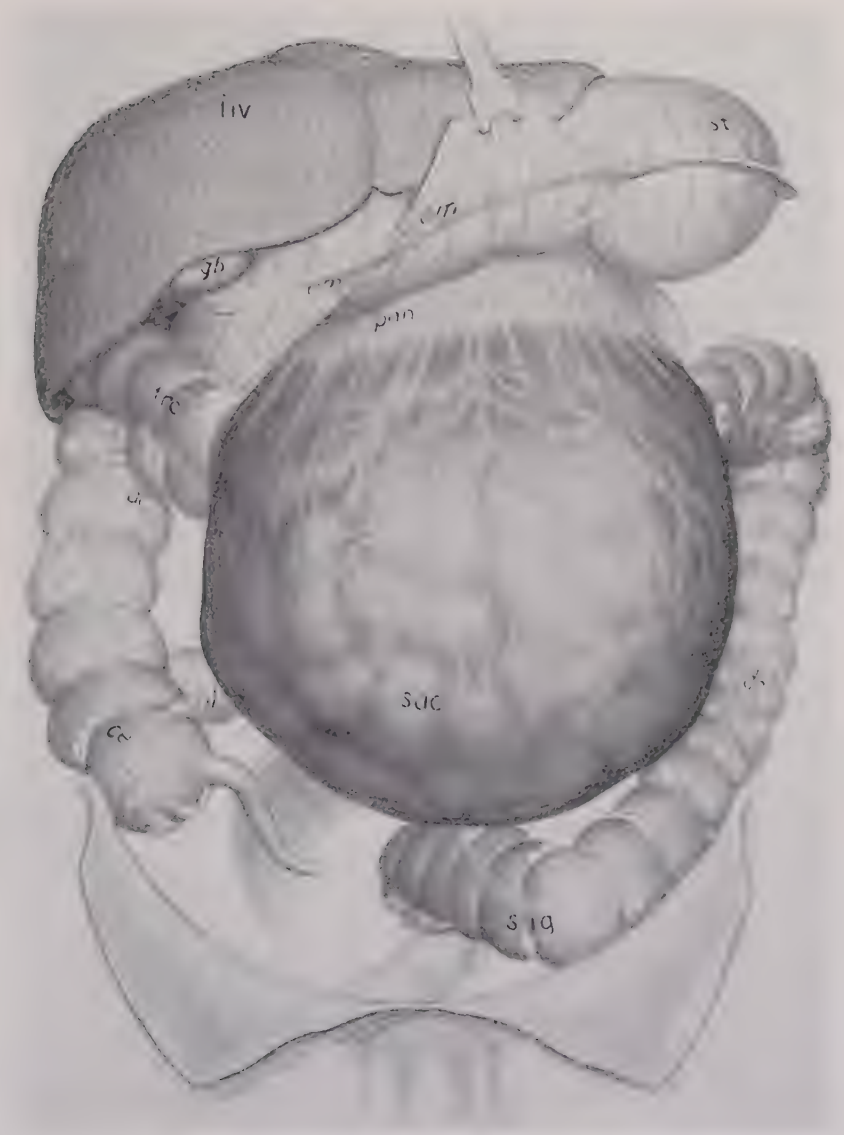

Fig. 2 A drawing of the abdominal viscera, with the greater omentum partly cut away and reflected to show the anomalous sac.

Abbreviations — Liv., liver; St., stomach; om., greater omentum; tr. c., transverse colon; ac., ascending colon; dc., descending colon; sig., sigmoid colon; cl., cecum; il., ileum; sac, anomalous sac.

verse colon which was in the sac measured 8 inches in length. The transverse mesocolon was short so that the transverse colon was nearly horizontal in position as it extended across the abdominal cavity. There were large holes in the transverse mesocolon both to the right and the left of the middle colic artery and between its branches.

The organs which normally lie against the posterior abdominal wall — the duodenum, mesentery and mesocolon — were normal and require no description. The superior and inferior duodenal fossae were shallow, but both were present. The paraduodenal fossa (Landzert) was absent, and there was no retroduodenal fossa.

There were no "open" passageways between the anomalous peritoneal sac and the abdominal cavity. However, there were 3 hiatuses in the sac and each transmitted the intestine. All 3 hiatuses lay against the posterior abdominal wall and were closed completely by the transmitted intestine. The positions of the hiatuses are seen in figure 2. The upper right hiatus is shown transmitting the transverse colon, as does also a hiatus on the upper left border of the sac. At the lower left border of the sac the ileum can be seen emerging from the sac.

DISCUSSION

Anomalous peritoneal sacs containing the mesenteric small intestine are almost always accompanied by extensive disarrangement of the colon, but the case described here possesses no complications. According to Moynihan ('06) the sac represents the hypertrophied wall of a fossa associated with the duodenojejunal junction resulting from a hernia of the small intestine into the fossa, and the disarrangement of the colon is only a sequela of the hernia. Treitz (1857) had earlier laid the foundation for this interpretation, and most of the recent writers have accepted this viewpoint. Such herniae have been called paraduodenal, retroperitoneal and internal. In spite of the fact that they are relatively rarely found and rarely, if ever, interfere with physiological action or terminate fatally, there are many cases reported in the

literature. The reason for this may lie in the fact that the sacs are clearly defined structures which are rarely encountered, and they seem to be easily explained.

Ziskind ('40) has reviewed 51 cases in which a peritoneal sac containing the small intestine was interpreted as a hernia into the mesentericoparietal fossa of Waldeyer, the identity of that fossa as the site of origin depending upon the relation of the colon to the sac (Desjardins, '18). These are called right paraduodenal herniae. Bryan ('35) has reviewed 162 cases of hernia which were supposed to have originated in the paraduodenal fossa of Landzert (the fossa between the inferior mesenteric vein and the left colic artery). This fossa is identified as the site of origin also because of the relation of the colon to the sac and such cases have been termed left paraduodenal hernia.

Actually, real evidence that any of these sacs are the result of a hernia into one of the fossa at the duodenojejunal junction is lacking and doubt concerning the validity of this interpretation has been expressed by many investigators, notably by Andrews ('23) and Nagel ('23). One of the great weaknesses pointed out by these investigators is the absence of a case that is incomplete or arrested at such a stage that there is irrefutable proof of the original place of herniation. The reason for this lack is more easily appreciated when it is realized that such sacs (or herniae) occur in considerably less than 1% of all bodies that come to a post-mortem examination, and that of these exceedingly few are foetuses.

Although most observers have in the past adopted Moynihan's explanation, there are others who have different but equally plausible interpretations. Hepworth ('16) believed that the sac developed not as a hernia but "as a result, perhaps, of an unusually long mesocolon, the umbilical loop upon returning to the abdominal cavity causes a lateral bulging of the mesocolon to the left, and this becomes the wall of the sac." Andrews ('18) believed the sac was a congenital anomaly due to the imprisonment of the small intestine beneath the developing mesocolon.

Papez ('32) believes both "right and left paraduodenal herniae and their surrounding sac have a more natural origin in the very early embryonic umbilical hernia. When the small intestines are drawn into the abdominal cavity, they also draw in with them their surrounding peritoneal umbilical sac due to a premature adhesion of its orifice to the duodenal coils and to the mesenteric root. In case the large intestine has been fully rotated to the right side across the duodenum and mesenteric root before the adhesion of the orifice of the sac and retraction of the gut had taken place, it would produce the more common form or left paraduodenal hernia in which the abnormal sac is situated to the left of the ascending colon and is suspended from the transverse colon and its mesentery. The cases of Desjardins ('18), Novak and Sussman ('24), and others are good examples. In case the large intestine has not completed its rotation before the adhesion of the orifice of the sac and retraction of the gut had taken place, the condition produced would be a right paraduodenal hernia, in which the abnormal sac is situated to the right of the ascending colon."

Conditions in the embryo are entirely adequate to account for the development of the sac to the satisfaction of each of the theories. However, there may be some conditions present which may give some indication of the mode and place of origin and other conditions which may cast doubt against the theory. If it is true, as most authors conclude, that the sac has its origin in a duodenojejunal fossa, then several questions such as Andrews ('23) raises must be answered. Why is the dorsal mesogastrium never included in the sac? Why are the transverse colon or descending colon rarely in the sac (Pybus, '16, and this report) and the descending colon never yet reported within the sac? How can the duodenojejunal junction ever retain its normal position? How can the neck of the sac be considered the opening when there are no blood vessels in the borders of the opening which were originally the boundary of the fossa? (Moynihan, '06; Desjardins, '18). It seems that

the theory of Hepworth, Andrews, and Papez explains these conditions fully as satisfactorily as the theory of Moynihan.

The specimen described in this paper is very clearly different from all others found described in the literature. In this specimen there was no abnormality in the position of the abdominal viscera, thus indicating that there was no interference with the normal rotation of the gut. This, in turn, implies that there were no adhesions between the wall of the embryonic umbilical sac and the intestine — connections which are believed to initiate the development of the anomalous peritoneal sac and cause a displacement of the colon. In a like manner of reasoning the lack of displacement of abdominal viscera makes it unlikely that the anomalous sac could have originated from a hernia into one of the paraduodenal fossae, or from the mesocolon. It is believed that the sac of this specimen originated from the dorsal mesentery of the stomach — specifically the posterior layer of the greater omentum and was due to an adhesion between that structure and the peritoneum of the posterior abdominal wall.

The presence of 3 openings into this sac (fig. 2) is interpreted as evidence that the fold of peritoneum which developed into this sac was a structure independent of the intestine to such an extent as to allow for the normal rotation of the intestine. Further strong evidence that this fold arose in the region of the dorsal mesogastrium is presented by the blood supply of the sac. The arteries and veins were, as previously stated, branches of, and tributaries to the splenic artery and vein. The smaller branches of arteries and veins from the inferior mesenteric artery and vein might well have been secondary vessels which invaded the sac wall at a later stage, after the fold from the mesogastrium had fused with the posterior wall of the abdomen.

In this specimen the usual fusion of the posterior lamella of the great omentum with the transverse mesocolon did not exist. This resulted in the presence of a peritoneal space between these 2 structures. The failure of these 2 folds to fuse might have been due to an accumulation of peritoneal fluid

between them. It is believed that the large holes found in the transverse mesocolon indicate that the amount of tissue normally contributed to this structure was impaired. It is not believed that the reduced mobility of the transverse colon accounts for the holes since the small intestine was similarly restrained in its movements, but its mesentery showed no such defect.

SUMMARY

A case is described in which the jejunum, ileum and transverse colon with their respective mesenteries are enclosed within an anomalous peritoneal fold which arose from the anterior surface of the pancreas and was fused with the posterior abdominal wall to form a sac. The sac resembles in general those described by previous authors, and most frequently interpreted as resulting from a hernia into one of the paraduodenal fossae. This specimen possessed features which clearly separates it from the category of a hernia. It is postulated that this sac had its origin from the dorsal mesentery of the stomach. This case description is the first in which the transverse colon was included in a peritoneal sac with the jejunum and ileum.

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HISTOLOGICAL STUDIES OF MUSCLE FROM CROOKED NECK DWARF FOWL

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EIGHT FIGURES

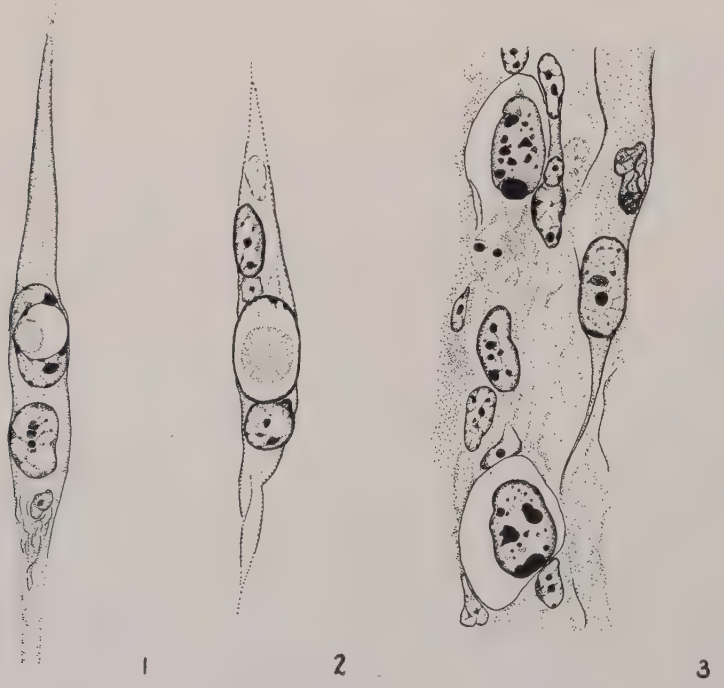
Asmundson ('45) has described a crooked neck dwarfism in one strain of the New Hampshire breed of fowl, and has found that the condition is due to an autosomal recessive factor. Effects of the latter become evident as retarded growth beginning with the thirteenth day of incubation. Affected embryos are stiff, with reduced muscle mass of the legs, wings, and neck, and in some cases have an abnormal sternal skeleton. The embryos are non-motile and unable to emerge from the shell; death often occurs before the twenty-first day. Similar conditions have been reported in other animals such as sheep (Roberts, '29), cattle (Hutt, '34), and swine (Hallquist, '33). Roberts suggests that wryneck of humans may be a comparable condition.

The pathologic findings in sheep by Roberts were described as muscle degeneration due to ischemic fibrosis of various groups of flexor muscles. Gruenwald ('44) described an hereditary microphthalmia of chick embryos in which development was normal during the first 5 days of incubation; changes toward the microphthalmic condition occurred late on the fifth day.

Dr. Asmundson has kindly furnished material from affected individuals of the New Hampshire strain described, for histological study of the muscle tissues. Samples from the neck and leg were taken from 19-day embryos. Fixation was best after Bouin's fluid. Gross examination of the embryos showed

that some muscle masses were normal, while others were much reduced in size. In the affected muscles the individual muscle fibers varied from a few that were normal to others which were stringy cells scarcely recognizable as muscle tissue. Hyaline degeneration was seen in some fibers but the greater part of the muscle showed other types of degeneration, principally loss of fibrils, loss of cytoplasm, and highly aberrant nuclear phenomena. In some cells, cyst-like structures (fig. 2) occurred. An earlier phase of the same development is shown in figure 1. The most striking nuclear phenomenon was that of large skeins (fig. 5). Initial stages of the skeining

Figs. 1–6 are of skeletal muscle cells from the tibial region of the leg of 19-day crooked neck embryos. Fixation, Bouin's fluid. Stain, Regaud's haematoxylin. Magnification approximately × 1260.



Figs. 1 and 2 Cyst-like formation in degenerating nuclei. Figure 1 also shows evidence of a skeining nucleus.

Fig. 3 Pycnotic nuclei and loss of cellular outlines.

process are seen in figure 4. It was not unusual to find both abnormal and normal-appearing nuclei in the same cell. The pycnosis of nuclei (fig. 6) was the only type of degeneration in which all nuclei of a particular cell appeared in the same phase. Loss of cytoplasm resulting in thin, tapering cells was of general occurrence, and the loss of cellular outlines was frequently seen (fig. 3). Another type of pycnosis (also in fig. 3), could not be traced in its earlier development; it appears related to the cyst type of degeneration.

The whole picture in the muscles is of a rapid progressive degeneration resulting in non-coordinating muscle masses; hence the immobile condition of the embryo at hatching. The skeining nuclei appear to be a type of spireme incapable of progressing further in mitotic activities. No mitotic spindles of muscle cells were seen in the affected muscle material studied, although dividing nuclei of muscle cells were seen in normal tissues from chick embryos of the stages indicated.

It is apparent that normal development of the muscle tissue had occurred in the earlier stages (? prior to the thirteenth day) and that degeneration was a subsequent development. The action of the genetic influence is comparable to that in the microphthalmic condition (Gruenwald, '44), in that both are deviations from an early normal development.

Unfortunately the material for the crooked neck studies has not been sufficiently plentiful so that the earlier phases of change from normal to abnormal could be detected. The stock of fowls with this factor is being increased so that a more thorough study of the problem can be undertaken.

Whether the degeneration noted is the result of an ischemia cannot be stated, but the condition is not a fibrosis as described for sheep by Roberts ('29). Fibrous tissue is present in our material, and is proportionately more abundant than in normal muscle, but the latter situation is due to the reduced amount of muscle cytoplasm rather than to an actual increase in the amount of fibrous tissue. If active fibrosis were occurring, numerous mitoses should have been encountered but only a few were seen in the connective tissue of both the normal and

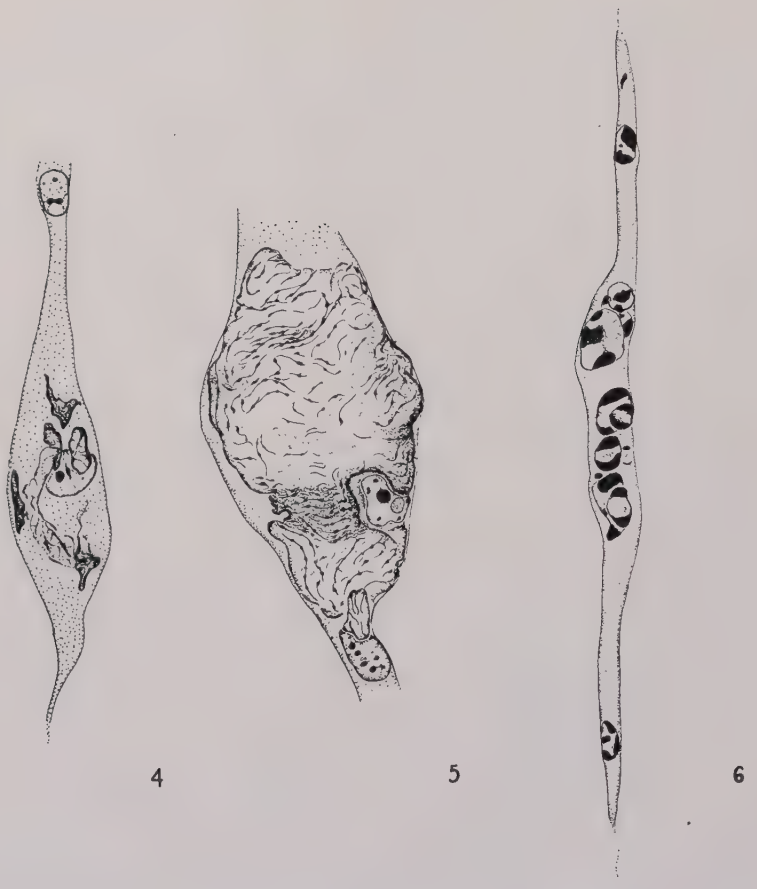


Fig. 4 Two normal nuclei and three in the skeining phase of degeneration.

Fig. 5 Late stage of skeining with two normal nuclei also present. In this case the huge skein is probably a composite of several nuclear contents.

Fig. 6 Simultaneous pycnosis of all nuclei in a single cell.

affected specimens examined, and hence can only be interpreted as normal multiplication of connective tissue cells, a further indication that the genetic action is specifically confined to muscle tissue.

The progressive diminution of the muscle masses due to degenerative processes described, simultaneous with continued growth in the skeletal framework of the neck, creates struc-

tural imbalance. This results in a twisting of the cervical column which is evidenced by the crooked neck condition.

The possibility that a genetic influence may also be reflected by changes in the circulatory and nervous systems must not be overlooked. Nerve preparations made by the technique of Bodian ('37), however, show a generous supply of afferent

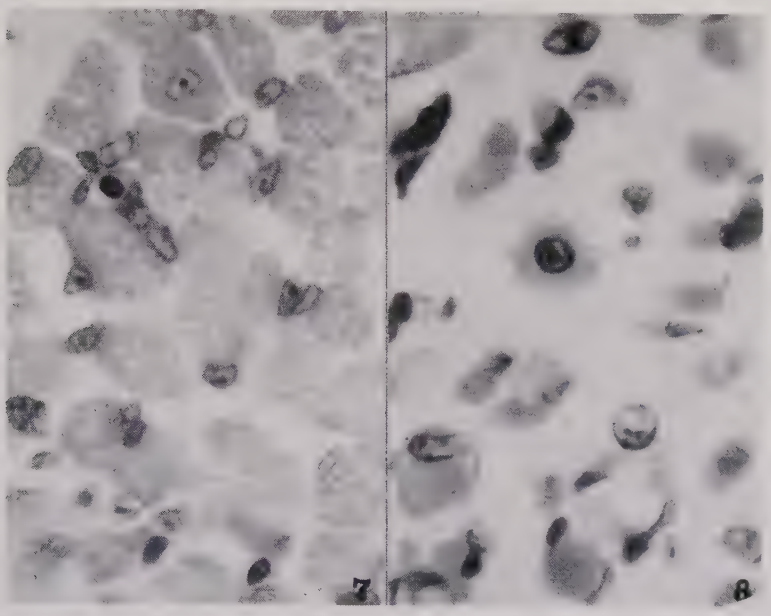


Fig. 7 Cross section of muscle of neck region of normal 19-day chick embryo. Magnification approximately × 1350.

Fig. 8 Cross section of muscle of neck region of 19-day crooked neck embryos showing extensive deviation and degeneration from the normal. Magnification approximately × 1350.

and efferent nerve mechanisms in the degenerate areas and the capillary supply to the affected muscle is sufficient to preclude an ischemic condition. Gross inspection of the skeleton of wings, legs, and neck in affected embryos show no obvious abnormalities. The present paper deals only with the end result, a degenerate and useless muscular system.

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BILATERAL RENAL AGENESIA

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SIX FIGURES

While investigating the development of the venous system an excellently fixed human embryo of 15.5 mm (crown-rump length) became available and was sectioned serially. Examination of the sections has revealed that the permanent kidney and the ureter are absent on both sides while the cranial portion of the Wolffian bodies and ducts are rudimentary, that is to say only some mesonephric tubules in this region open into the Wolffian duct. Also the Müllerian duct is demonstrable for only a few sections beyond the abdominal ostium. At the level of the superior mesenteric artery both mesonephric ducts have disappeared and on the right side only a few abnormal mesonephric glomeruli are present. Caudal to the level of the artery only the gonads have differentiated in the urogenital fold. These glands show normal development in all sections and already have the structure of testicular tissue.

The blood vessels in these regions differ from the normal in the respect that the mesonephric vessels are reduced in size. However the caudal part of the postcardinal veins, the subcardinal veins and the azygos veins show normal relations, this observation being of importance with regard to the interpretation of the inferior vena cava and of the migrations in the dorsal body wall (Auër, '46).

In order to describe this anomaly adequately, it is necessary to compare this with certain sections of a normal embryo

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of about the same stage of development (Embryo VII of our collection, measuring 18 mm crown-rump length). Three different levels have been chosen for this purpose namely the levels of the suprarenal gland (Th. 11), of the intersubcardinal anastomosis (L1) and of the aortic bifurcation (L3).

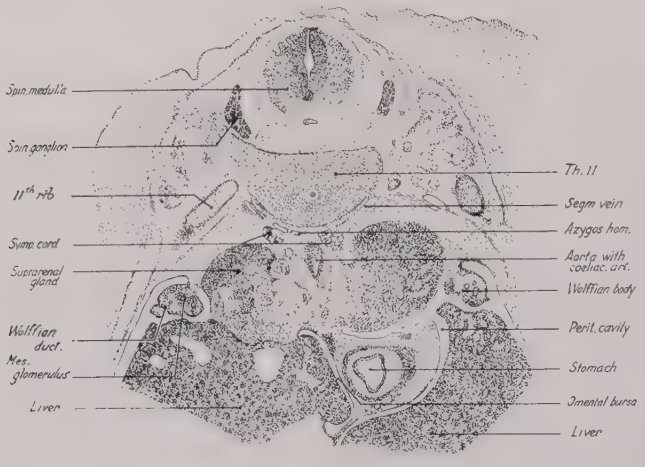

Fig. 1a Transverse section of the abnormal embryo in the region of the Suprarenal gland.

Fig. 1b Transverse section of the normal embryo in the region of the Suprarenal gland.

Figures 1a and 1b demonstrate a transverse section through the cranial part of the mesonephros in which mesonephric glomeruli, the Wolffian and Müllerian ducts may be seen. Only slight differences in the mesonephric tubules and glomeruli are demonstrated at this level. In the 15.5 mm specimen the mesonephros possesses a smaller number of glomeruli

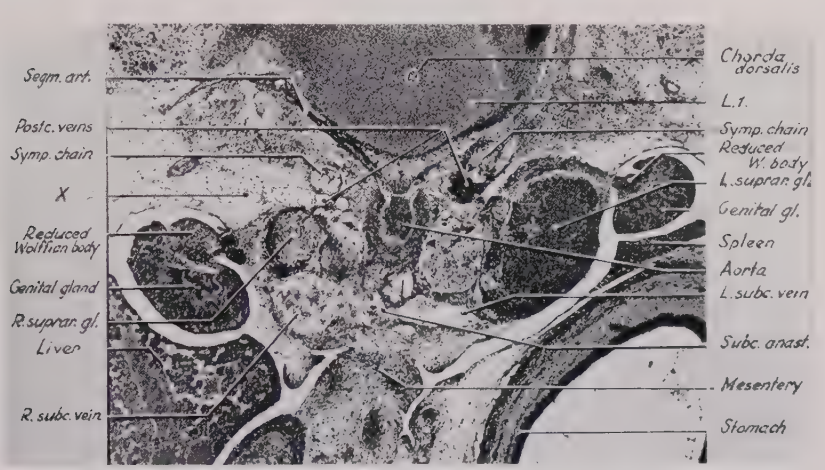

Fig. 2a Transverse section of the abnormal embryo in the region of the Subc. anastomosis.

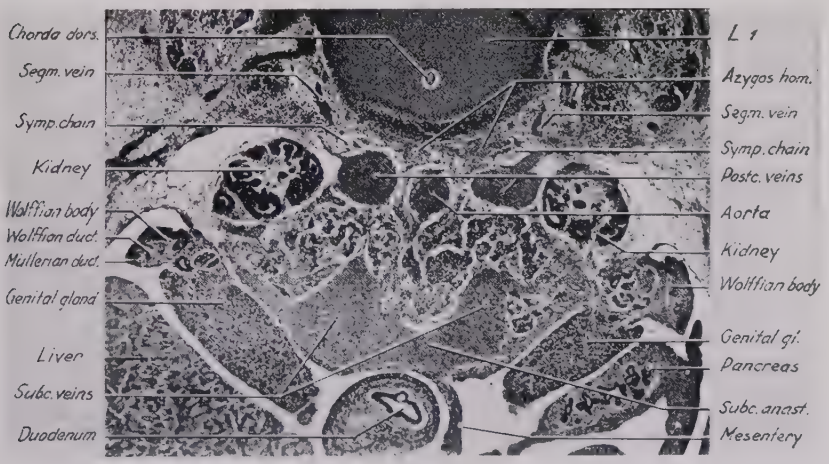

Fig. 2b Transverse section of the normal embryo in the region of the Subc. anastomosis.

and tubuli. One of these abnormally large glomeruli is seen in the right mesonephros of figure 1a. The great vessels (subcardinal and thoracic azygos veins) show the same differentiation in both embryos.

On the level with the intersubcardinal anastomosis (figs. 2a and b) a great difference is observable in the kidney of the 2

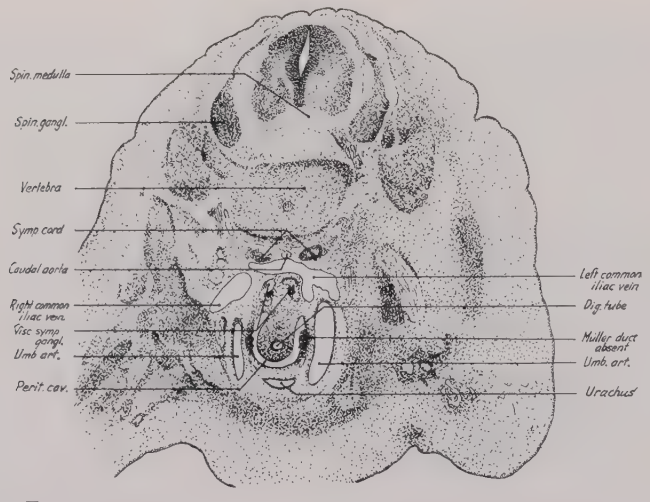

Fig. 3a Transverse section of the abnormal embryo in the region of the aortic bifurcation.

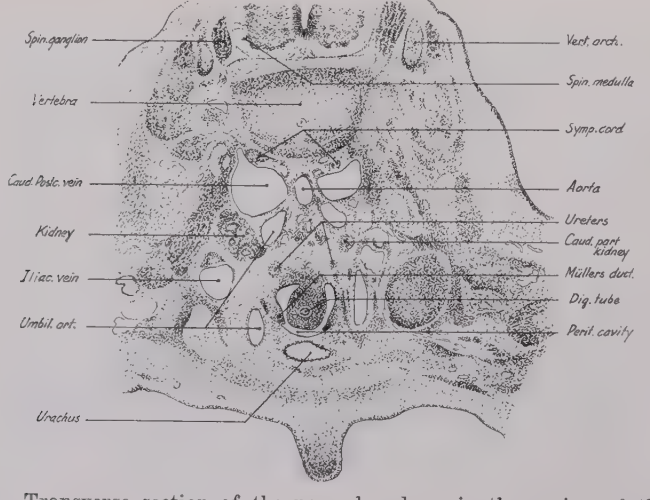

Fig. 3b Transverse section of the normal embryo in the region of the aortic bifurcation.

specimens. In figure 2a, the X shows the empty space in which the kidney should be situated (cf. fig. 2b). As the section in figure 2a is not quite a transverse section the cauda of the suprarenal gland is smallest at the right side. Figure 2b shows normal Wolffian bodies while in the anomalous specimen this organ is much reduced in size. An abnormal glomerulus is seen at the right side while the Wolffian and Müllerian ducts have disappeared. The genital glands do not show any difference in the 2 figures, which is also the case with regard to the postcardinal veins. The smaller diameter of the right postcardinal vein (in the thoraco-lumbar line) does not justify the possible conclusion that this vein is influenced by the anomaly, since in other sections the vein has a larger diameter.

Finally, sections in the region of the aortic bifurcation, as illustrated in figures 3a and 3b, show the absence of the Müllerian and Wolffian ducts in the abnormal embryo. It is to be noted that the section illustrated in figure 3a has not exactly the same direction as the one in figure 3b and therefore the aortic bifurcation does not show the same relations.

DISCUSSION

It is believed this case may be worth reporting since agenesia of *both* permanent kidneys has only been described once in a human embryo (Gruenwald, '39). Unilateral agenesia in human embryos has been found by Kornfeld ('26) in a fetus of 5 cm and by Boyden ('32) in an embryo of 10 mm. Ballowitz (1895) reviewed the whole literature on congenital absence of the kidney in adults. He mentioned the congenital absence of 1 kidney only and distinguished 3 types of abnormalities.

1. Pseudo-aplasia due to concrescence of both kidney-rudiments.
2. Partial aplasia of the kidney.
3. Total aplasia of the kidney.

He emphasizes the difference of frequency in both sexes. The ratio in his material is 113 for man and 71 for women. In

males the abnormality is more often found on the left side, while in females no special side is preferred. According to Kermauner ('24) unilateral agenesia of the kidney occurs in 1 or 2 cases in every 1000 autopsies. Ballowitz stresses the fact that in females the anomaly is more often associated with abnormalities of the genital tract, although the genital gland always remains intact. He finally suggests that the accompanying abnormalities will also be discovered in males, though not so easily, in consequence of difficult exploration. These associated abnormalities are reported by Kornfeld in a 5 cm fetus in which the kidney, ureter, and the greater part of the Wolffian body are absent on 1 side of the body. Also the Müllerian and Wolffian ducts are lacking in the region where the Wolffian body is not developed.

In adult cases the typical anomaly in males shows, besides unilateral absence of the kidney, an aplasia of the ductus deferens and of the greater part of the epididymis. The caput of the epididymis as a rule has been differentiated in consequence of the fact that the cranial part of the mesonephros does not take part in the agenesia, as is also seen in our embryo. As the urogenital ridge is normal the descent of the testis does not show any abnormal course. Of course the absence of the Müllerian duct has no consequence in males. In women this abnormality leads to a uterus unicornis and a unilateral agenesia of the tuba uterina. To summarize the above described facts it may be said that renal agenesia is almost always accompanied by other abnormalities. In animals the anomaly is sometimes accompanied by agenesia of the lower extremity, as is also reported by Krediet and Schultze ('25).

Regarding the causes of all these embryological disturbances many possibilities have been suggested. A reduction of the umbilical artery has often been thought responsible, being a primary cause for the absence of the vessels to the kidney. As the vessels play rather a passive role in ontogeny it is believed that they are not responsible for these extensive anomalies. In the above described embryo the umbilical ar-

teries are normal on both sides. Accordingly, this theory may be discarded.

Brown ('31) concluded that a strain of mice derived from a previously x-rayed pair, showed a retardation of the development in the region of the urogenital ridge which lessens the contact between the ureter and the kidney. In consequence of this process the kidney may be poorly developed. Also Fischel ('12) emphasized the importance of a contact between the ureter and the kidney in order to induce a differentiation of the metanephrogenic blastema.

Boyden ('24–'32) experimented with chick embryos of 2 days. He damaged the growing Wolffian duct by electrolyzing its caudal end. After the experiment the eggs were closed again by thin mica plates so as to make life possible until the seventh day. At this time, or earlier, the thus prepared embryos were fixed and cut in serial sections. If the obstruction was placed cranially of the growing end of the Wolffian duct the sections demonstrated an interruption in its course and hydronephrosis of the Wolffian body in the region above the lesion. An interference at the growing point brought the following abnormalities.

a. The duct was absent below the lesion.
b. The Wolffian body was intact only in the region of the undamaged duct, i.e. above the lesion.
c. No permanent kidney appeared because there was no distal Wolffian duct from which the ureteric bud could develop.

These observations were in complete accordance with the descriptions of Kornfeld's fetus, while Gruenwald ('37) after similar experiments produced similar anomalies. It is for this reason that Boyden and also Gruenwald conclude that the Wolffian duct induces the development of the Wolffian body, while the ureter induces the development of the kidney. Boyden sees a gradual reduction of the metanephrogenic tissue after lesion of the lower end of the Wolffian duct. No tubuli and glomeruli develop and soon the organ disappears as a whole. No aplasia of the metanephros is responsible for this

fact, a conclusion also emphasized by Gruber ('27). The rudimentary pronephros is considered to be a vital organ (Boyden, '27) for without the Wolffian duct, which is induced by this organ, neither the mesonephros nor the metanephros develop. It is noteworthy that the experimental hypertrophy as seen by Boyden also occurs in the anomaly (fig. 1a) which certainly proves the value of the experiments. Besides the already published data of Kornfeld, Boyden and Gruenwald, our own embryo, therefore, gives further evidence of the conclusions drawn by the above described experiments. The published embryos show the agenesia in different stages of development and the highest degree of abnormality hitherto described in this region is seen in our specimen.

Finally Gruenwald suggests that the contact of the ureteric bud with the kidney-rudiment must take place in a limited space of time, because of the cranio-dorsal migration of the kidney. As soon as this movement has started, the ureter could not possibly reach the metanephros, in consequence of which the reduction of the kidney would set in.

It seems very doubtful whether the mesonephroma ovarii of Schiller ('39) may be a result of the inducing power of the Wolffian duct in the region of the genital gland, as is suggested by Schiller and also by Gruenwald. This tumor according to Kazancigil c.s. ('40) seems to be a papillo-endothelioma ovarii. The evidence they give against the conclusion of Schiller may be accepted, since their reconstruction of a part of the tumor does not show any glomerulus-like structure. In addition to this type, other anomalies have been described in which the gonad also is underdeveloped (van den Broek, '07); also Priesel ('24) published a paper, dealing with a hypoplastic Wolffian duct and a normal Müllerian duct resulting in a certain degree of pseudohermaphroditismus masculinus. All these abnormalities diverge from the picture described above in which the primary cause must be sought in an agenesia of the Wolffian duct with secondary agenesia of the Wolffian body, the ureter, the kidney and consequently of the epididymis and the spermatic duct.

Finally the question arises whether the gonads are independent of this process of induction and if so what may be the cause of their agenesia in the rare cases in which they are also absent. The anomalous embryos of this kind hitherto described provide sufficient evidence for the conclusion that the development of the gonads is influenced by other factors, a conclusion supported by the experiments mentioned above. These other factors are not known but the combination of absence of gonads with the picture described in our own specimen seems to suggest that these factors occur in a younger stage of development. Van den Broek has already stressed the fact that the total absence of the urogenital system is very seldom seen in comparison to anomalies of the uropoietic system only.

SUMMARY

1. Another case of bilateral renal agenesia in a human embryo (15.5 mm) is described and the explanation of earlier investigations continued.

2. It is doubted whether the mesonephroma ovarii of Schiller really consists of induced mesonephric tissue.

3. The factors causing anomalies of the whole urogenital system are briefly discussed.

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NATURE OF FUNCTIONAL RECOVERY FOLLOWING REGENERATION OF THE OCULOMOTOR NERVE IN AMPHIBIANS

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There is a striking difference between the type of motor recovery which occurs in mammals and that which occurs in amphibians following regeneration of divided limb nerves. The abnormal connections formed between central nervous system and musculature by the inevitable misdirection of regenerating motor axons leads in mammals to confused and incoordinate mass contraction of the reinnervated muscles (Sperry, '45c). In amphibians such misregeneration is followed instead by recovery of normal motor coordination (Weiss, '28, '36, '41). Evidently in amphibians the central discharge of the regenerated motor neurons somehow becomes adjusted to suit the particular limb muscles in which the outgrowing axons happen to terminate, but this kind of readaptation does not take place in mammals.

The following experiments were undertaken to find out if the same difference between mammals and amphibians exists with respect to the effects of regeneration of the third cranial or oculomotor nerve. The results of regeneration of this nerve in man have already been amply described (Bender and Alpert, '37, Bielschowsky, '40, Wartenberg, '46) and show that motor readjustment fails to occur, as is characteristic of mammalian nerves generally. There is recovery only of an un-

[1] A major portion of the work was carried out at the Yerkes Laboratories of Primate Biology in Orange Park, Florida. It was also supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

differentiated mass contraction of the various muscles supplied by the regenerated oculomotor nerve and this persists permanently without correction. Similar effects have been reported for the chimpanzee and monkey (Bender and Fulton, '38, '39). It only remained for the above purpose therefore to determine whether in amphibians regeneration of the oculomotor nerve would lead to abnormal mass contraction as in man, or whether it would lead to restoration of normal motor coordination as does the regeneration of limb nerves in amphibians.

The problem is significant for a number of reasons. First, the results have important implications regarding the nature of central nervous integration. The recovery of normal coordination despite the mix-up of nerve connections in the limbs has been interpreted to mean that our traditional connectionist theory of central nervous organization is inadequate and must be radically revised (Weiss, '28, '36, '41). It has also been taken alternatively to mean that the normal growth of central synaptic connections is regulated through induction effects from the peripheral end organs (Sperry, '41, '43). In either case the implications are significant and if the latter view is correct, the results assume special interest for their bearing on the ontogenetic development of central reflex associations. The question has relation likewise to problems of reeducation following disarrangement of nerve connections. There is particular interest in the oculomotor nerve in this regard because it is in the ocular motor system that the evidence to date most strongly favors the possibility of immediate spontaneous readaptation of basic neuronal relations (Marina, '15; Leinfelder and Black, '41, '42). The value of advancing the experimental analysis of the vestibulo-ocular system from all possible angles has been pointed out elsewhere (Lorente de Nó, '33; Sperry, '46) and accordingly the data take on added significance by virtue of their application to the motor portion of the vestibulo-ocular reflex arc. Already the analysis of the structure and function of this particular reflex system, contributed in large share by the work of Lorente de Nó, repre-

sents the most advanced picture we have of the more refined aspects of central nervous integration.

It was in the hope of gaining further insight regarding the development of appropriate central linkages between the various neuron elements of the vestibulo-ocular system that the following experiments were started. In regeneration of the oculomotor nerve just as in the initial outgrowth in development there arises the problem of adjusting the central and peripheral relations of the individual neurons in a systematic manner so that they become mutually suited to each other and adapted for normal function. The outgrowing axons which terminate in the inferior oblique muscle, for example, must have special functional associations in the nerve centers quite different from those of axons which terminate in the superior rectus muscle or in the medial or inferior rectus muscles, etc. Where such adjustment is attained in amphibians after nerve regeneration, particularly in young larval stages such as are used in the following study, the adjustment presumably involves processes similar to those which operate in normal development.

Previous experiments have shown that the ontogenetic structuring of the vestibulo-ocular reflex mechanisms is accomplished by the growth process itself without aid of learning (Nasiel, '24; Mowrer, '36; Sperry, '46). It has been shown further that in centripetal regeneration of the eighth cranial nerve the different types of sensory fibers establish synaptic relations in the vestibular nuclei in a discriminative manner to suit in each case the particular end organs with which they are connected in the periphery (Sperry, '45b), indicating that the selective linkage of neurons on the sensory side is made possible by differential affinities between the different types of central and peripheral neurons. The present work was begun in search of evidence of the existence of similar specificity and selective affinities on the motor side of this same reflex arc.

PROCEDURE

The plan of the experiments was to sever completely the oculomotor nerve in a rough manner so as to preclude any mechanical guidance of individual fibers back into their original channels. The type of motor recovery which ensued was then to be compared with that known to follow complete interruption of the same nerve in man. The outcome of the initial experiments indicated a necessity for various additional operations and control measures, the nature and purpose of which are explained separately in their contexts.

The compensatory vestibulo-ocular reflexes were used entirely in estimating recovery of function. Amphibians do not make voluntary exploratory eye movements to any appreciable extent nor is the upper lid actively retracted as it is in man. The compensatory eye movements were elicited by tilting the animals in the 3 primary planes of the body. These responses and the method of observing them under a stereoscopic microscope have been described previously (Sperry, '46). It is necessary here, however, to define the terms used below in describing the eye movements in the different body planes. Movement of the eye on its dorsoventral axis in the horizontal plane is referred to as *horizontal movement.* The horizontal rectus muscles, i.e. the medial and lateral rectus muscles, are primarily involved. Movement of the eye around its optic axis is referred to as *wheel movement.* This is effected primarily by the oblique muscles. The eye is said to wheel forward when the dorsal pole of the globe rolls anteriorly and to wheel backward or caudally when the dorsal pole rolls posteriorly. (It should be remembered in visualizing these eye movements that the eyes in these animals are placed laterad.) Movement of the eye around its anteroposterior axis in the transverse vertical plane is called *vertical movement.* Movement in this plane is effected primarily by the vertical rectus muscles, i.e. the superior and inferior rectus muscles. The vertical movements are most pronounced, the horizontal least so.

The extrinsic eye muscles supplied by the oculomotor nerve in amphibians and paralyzed by its severance are: the inferior, superior, and medial rectus muscles and the inferior oblique. The superior oblique muscle was also routinely paralyzed in the main experimental groups by excising a long stretch of the trochlear nerve. This left unparalyzed only the lateral rectus and retractor bulbi muscles and thus virtually abolished all wheel and vertical movements of the eyeball. Any consistent recovery of either vertical or wheel movements in the proper direction as a result of regeneration of the third nerve under these conditions would therefore mean either the presence of selective factors operating in regeneration or else some type of subsequent functional readaptation neither of which appears in man or the chimpanzee.

The animals were frog tadpoles *(Rana clamitans* and *R. grylio)* and salamander larvae *(Amblystoma talpoideum)* gathered in northeast Florida in the spring and early summer. Unless otherwise indicated the tadpoles were in mid- and late larval stages ranging from about 3 to 6.5 cm in length. The amblystoma were also in mid- and late larval stages ranging from 4 to 7 cm in length. The eye movements are decidedly more conspicuous in the tadpoles than in the salamander larvae and somewhat more pronounced in *R. grylio* than in *R. clamitans.*

Anesthetization was carried out in a 2.0% water solution of urethane and the operations were done in a 0.5% water solution of urethane. In the tadpoles a tongue-shaped flap of skin was cut free and folded forward off the cranium. After operation the incision was closed by simply folding this flap of skin back into position. In the urodeles a straight longitudinal incision was made, the edges of which were later approximated as far as possible. The cranial wall over the mid-brain was punctured and broken away in pieces with no attempt to replace it. A fine hook the vertical movement of which was governed by a screw was employed for retraction of the optic lobe. A small suction pipette was used to clear away any blood which escaped into the operating field. Unless other-

wise mentioned the nerves were divided intracranially. They were not cut but were purposely pulled, pinched, and teased apart with fine pointed forceps in a way that left the ends of the nerve stumps frayed, ragged, and well separated. There was no chance under these conditions for the different fiber types to be routed back by mechanical factors alone into their proper pathways in the distal stump. Other technical details having more restricted application are given in their specific contexts.

OBSERVATIONS

Group 1. Oculomotor nerve broken, trochlear nerve widely excised

Almost the entire intracranial length of the trochlear nerve was extirpated. The oculomotor nerve was then divided about halfway between its exit from the brain and its passage through the cranial wall. Twenty-three animals were operated upon, 16 *R. grylio* and 7 *R. clamitans*.

The immediate functional effect of this operation, which paralyzes both oblique muscles plus the superior, inferior, and medial rectus muscles, was to eliminate all vertical and wheel movements of the eye. Only movements in the horizontal plane remained and their excursion was reduced. These horizontal movements were mediated by the lateral rectus muscle the innervation of which remained intact. The unopposed tonic action of this same muscle caused the resting posture of the eye to be deviated temporally.

The first signs of functional recovery occurred about the fifth day after operation when wheel movements began to reappear. These were not normal wheel movements, however, because they were elicited by stimulation which in the normal animal produces only vertical movements nor did they occur in response to stimulation appropriate for wheel movements. Tilting the animals on their longitudinal body axis in either direction from the resting position caused the eye to wheel caudalward on its optic axis. As the head was returned to the level resting position, the eye rolled rostrally again. In the

next few days these wheel movements became stronger and in addition a horizontal component entered the response in that the nasal pole of the eye pulled into the orbit slightly as the eye wheeled caudally.

The above type of reaction, i.e. wheel movement in the caudal direction accompanied by some medial deviation, is just what would be expected from mass contraction of the oculomotor musculature. One would anticipate the wheel component of the reaction to be most conspicuous because the muscle primarily opposing this movement, the superior oblique, had been completely paralyzed by extirpation of the fourth nerve. The medial deviation would be expected to be present but less strong than the wheel component because the action of the muscle responsible, the medial rectus, was opposed by the normal tonus of the intact lateral rectus and also because the normal excursion of movements in the horizontal plane is relatively small. Little or no vertical component was evident in the response. This would be anticipated because the antagonistic actions of the superior and inferior rectus muscles would tend to cancel each other.

Rotation of the animals on their transverse body axis or rotation on their dorsoventral body axis was not so effective in eliciting the mass reaction. This may be attributed to the fact that these stimuli do not normally activate nearly so large a percentage of the oculomotor fibers as does rotation on the longitudinal axis. In some cases a slight caudally directed wheel movement could be elicited by rotation on the transverse axis but this was relatively slight compared to the response to rotation on the longitudinal axis. In the younger animals the abnormal wheel reaction was less marked, perhaps in part because the eye in these cases was already wheeled somewhat caudally in its resting posture.

Between the eighth and fifteenth days a vertical component began to be evident in the response to rotation on the longitudinal axis. The eye deviated downward when its side of the head was tilted upward and vice versa as is normal. These reactions, unlike the above, were selective and adaptive in

nature. The response was not always in the same direction but was directed dorsally when the head was tilted ventrally and ventrally when the head was tilted dorsally from the resting position. The excursion of these properly directed vertical movements, however, was consistently less than normal with considerable variation in different individuals. In some cases the vertical response remained so slight as to be just discernible while in the other cases it became conspicuous and approached about half the normal excursion. There was a tendency for the vertical movements to be recovered a little more completely in the younger larvae and for their recovery to be better in *R. clamitans* than in *R. grylio*.

An inward deviation of the dorsotemporal pole of the eye eventually appeared also in most cases when they were tilted tail up on the transverse body axis. This reaction was found to be present in animals with both oblique muscles denervated and was therefore considered to be further indication of proper action of the rectus muscles.

There was not much additional improvement in the ocular reactions beyond the stage of recovery reached at the end of the third week although half of the animals were kept until metamorphosis. After the third week the eyeball in the majority of cases began to sink into the orbit gradually as if pulled medially by constant contracture of the reinnervated muscles. There was a correlated reduction in the excursion of the ocular reactions. Because the excursion of the eye movements in anurans becomes greatly reduced at metamorphosis no attempt was made to follow the recovery of function beyond this point.

In 4 tadpoles the regenerated third nerve was resected following the recovery of adaptive vertical movements. This again abolished all vertical and wheel movements of the eye, normal as well as abnormal, leaving only horizontal movements mediated by the intact lateral rectus muscle. This ruled out any possibility that the ocular movements attributed to regeneration of the third nerve might have been due instead

to adjustments in the residual function of the lateral rectus and retractor bulbi muscles.

In 5 animals which were prepared for histological examination by the Bodian method it was possible to trace the regenerated third nerve peripherally into the oculomotor muscles. Exact fiber counts were not made but the number of fibers in the regenerated nerve distal to the region of transection was approximately the same as or only slightly less than that in the normal nerve of the opposite side. There was no extreme tangle of fibers in the scar region nor signs of excessive branching. It was difficult, in fact, to determine exactly where the nerve had been divided.

To summarize, the results in this group of animals indicated that the misregenerated fibers, for the most part, had retained their original central reflex timing without adjustment to the new motor terminations. This would account for the abnormal wheel movement with medial deviation and for the gradual retraction of the eye into the orbit, all of which suggest mass, undifferentiated contraction of the oculomotor musculature like that found to follow regeneration of the divided oculomotor nerve in mammals. There was evidence however, in the partial recovery of movements in the vertical plane of an abortive tendency for selective restoration of proper muscle coordination such as is found to follow regeneration of limb nerves in amphibian larvae.

There are several reasons why one might expect an incomplete functional readaptation, if present, to show up better in vertical movements than in either the wheel or the horizontal reactions. In the first place the normal excursion of the vertical movements is greater than that of the wheel and especially that of the horizontal movements. Secondly, there are 2 oculomotor muscles involved instead of 1 with the action of each tending to reinforce that of the other, so that the chances of slight partial recovery becoming manifest in the vertical plane are thus double those in the other planes on this count alone. Finally, the mass action of these 2 muscles would tend to be cancelled out because the 2 are antagonists. This

would permit any minor action in the correct direction superimposed upon the mass contraction to register an observable effect. On the other hand, the mass contraction of the inferior oblique and of the medial rectus muscles was not opposed by any muscle reinnervated by the oculomotor nerve, that of the medial rectus being opposed only by the normal tonus of the lateral rectus, while that of the inferior oblique remained without any opposition whatever.

Group II. Cross union of the trochlear to the oculomotor nerve

To test further the deduction that the misregenerated fibers retain their original timing as indicated by the results in group I, the central end of the divided fourth nerve was crossed to the distal end of the divided third nerve. Under these conditions the oculomotor muscles should become reinnervated by fibers of the superior oblique muscle and should thereafter contract in mass whenever the superior oblique normally responds. One would anticipate the mass reaction to be weak because there are so few fibers in the fourth nerve. Even so it seemed possible that the action of the inferior oblique muscle with no opposition to overcome might register observable wheel movement. Accordingly the operation was carried out in 16 tadpoles, 10 *R. grylio* and 6 *R. clamitans*. The oculomotor nerve was broken where it enters the midbrain and a short length of the stump was removed. The trochlear nerve was broken as far distally as possible and the end pinched against the end of the oculomotor nerve. The ends of the 2 nerves were held thus in contact or close together until they became fixed in place by coagulation of the surrounding media.

The immediate paralytic effects of this operation were similar to those of the preceding series. Between the sixth and eleventh day after operation 11 of the animals began to display wheel movements in the reverse direction from normal. When the tadpoles were rotated head up on their transverse

body axis, the eye rolled backward in its orbit instead of forward as in normal animals. And conversely, as the head was lowered the eye rolled forward again. Some wheel movement also occurred when the animals were rotated on their longitudinal body axis. The eye rolled forward when its side of the head was tilted upward and the eye rolled backward when its side of the head was tilted downward. Slight medial deviation apparently from action of the medial rectus muscle was also noted in most of the cases when the eye rolled backward in response to rotation on the transverse body axis. In the remaining 5 animals the nerve cross was apparently unsuccessful for there was no appearance of reversed movements. In 3 of them weak movements similar to those obtained in group I began to appear after a delay of 9 to 14 days as if the third nerve fibers had succeeded finally in regenerating into their own distal stump.

In 2 of the successful cases the reversed wheel movements persisted with little change. In the others the excursion of the wheel movements diminished gradually after about the tenth day due apparently to conflicting mass contractions resulting from regeneration of the oculomotor nerve which was only delayed rather than completely blocked in these cases. Severance of the regenerated third nerve without damage to the crossed fourth nerve in 2 of them resulted immediately in release again of excellent wheel movements in reverse. Later severance of the crossed fourth nerve in these 2 cases abolished the reversed wheel reaction. The reversal in the timing of the inferior oblique after its reinnervation by fibers from its antagonist in these cases was further evidence that fibers which have regenerated into foreign muscles may retain their original central excitation phase.

The persistence in this group of wheel movements in reverse direction shows that in the amphibians at least the central coordination patterns for eye movement are not immediately readapted to suit extreme peripheral rearrangements as implied by experiments on the transposition of eye muscles in the monkey (Marina, '15; Leinfelder and Black, '41, '42).

Group III. Branches of nerves III, IV, and VI broken separately in the orbit

It is contended by Wartenberg ('46) that the primary cause of mass movements following nerve regeneration is not the misdirection of regenerating fibers as commonly supposed but rather the damaging effect of the peripheral trauma on the central coordinating mechanisms. Division of the individual branches of the oculomotor nerve in the orbit in such a way that the regenerating fibers reach their own distal stumps rather than channels to foreign muscles should and does lead, according to Wartenberg, to mass movements just as does more proximal division with fiber misdirection. The present group of cases was prepared as a test of this point and also as a control for the preceding series to find out if the ocular muscles could function properly after regeneration of the third nerve, provided proper connections were restored. In 12 animals, all *R. grylio,* the roof of the orbit was opened widely and the individual nerve branches to each of the 6 main ocular muscles was divided separately producing complete immobility of the eye.

The results of nerve regeneration in these cases were strikingly different from those obtained in the first series in which there was opportunity for fiber misdirection. Between the fourth and eighth days following operation excellent ocular movements in all 3 planes had been reestablished in 7 of the 12 cases. In the extent of the excursion and in direction and timing the movements approached very closely those of the normal animal. The remaining 5 cases showed good recovery of movement in 1 or 2 planes with only partial recovery in the others, indicating that in these few instances the regenerating axons had not succeeded in reaching their appropriate distal stumps. The rapid restoration of adaptive movements with their full excursion in this group demonstrated that excellent functional recovery occurs when the regenerating nerves terminate in their proper muscles. These results in correlation with those of the preceding series are

opposed to the contention of Wartenberg and support the traditional assumption that the mass movements following oculomotor nerve regeneration are attributable to fiber misdirection.

Group IV. Third nerve broken, fourth nerve widely excised in Amblystoma

On the chance that functional readaptation might be more complete in urodeles, the tissues of which tend generally to maintain a greater embryonic lability than those of anurans, the oculomotor nerve was broken in a series of salamander larvae. The operations were performed through a single longitudinal incision; otherwise the surgical technique was much the same as that described for the tadpoles including extirpation of most of the intracranial length of the trochlear nerve.

The type of recovery produced by regeneration of the oculomotor nerve in these urodeles was very similar to that observed in the tadpoles with respect to wheel and vertical movements. Movements in the horizontal plane were not of sufficient magnitude to be tested, even in the normal salamander. The outcome in the urodele differed from that already described for the tadpole in 2 respects: First, the normal excursion of the adaptive vertical movements was more completely restored. The recovery was better in a relative sense only, for the normal excursion of these eye movements in the urodele is much less than in the tadpole. Secondly, wheel movements of almost normal character also were recovered in 7 of the 11 animals. This was apparently due, however, not to the recovery of function of the inferior oblique muscle but instead to regeneration of the trochlear nerve to the superior oblique muscle. Splitting the midbrain roof in the region of the decussation of the fourth nerves abolished the recovered wheel movements in 5 of the animals so treated while leaving reduced wheel movement on the contralateral side where the third nerve had remained intact. It is remarkable that the regenerating fibers of the fourth nerve were able properly to

span so great a distance and arrive correctly at their former termination. As in the tadpoles resection of the regenerated third nerve again abolished the recovered movements in the vertical plane. The tendency toward restoration of proper motor function may have been more pronounced in these animals than in the tadpoles as far as the vertical rectus muscles are concerned. Recovery was not noticeably better, however, in the case of the inferior oblique muscle. The same abnormal wheel component in the response to rotation around the longitudinal body axis appeared and persisted without correction. It was evident that in urodeles as well as in anurans, oculomotor fibers which have misregenerated into a foreign muscle may excite the new muscle without an adaptive change in the central timing of their discharges.

Group V. Regeneration of the oculomotor nerve in very young tadpoles

Since the functional readjustment which follows nerve regeneration in amphibians appears to be essentially developmental in character, the chances of obtaining such functional readaptation should be better, the younger the animals. Accordingly the experiments were repeated on a group of 9 very small tadpoles (all *R. grylio*) about 18 mm in length. The compensatory ocular movements were already present at this stage although their excursion was still relatively small. As in groups I and IV the third nerve was broken and the fourth nerve excised intracranially. A single longitudinal incision was used as in group IV. The fourth nerve was so fine in these very young animals that it was not seen in most cases. It was broken by passing forceps through its pathway between the brain and cranial wall but to what extent the attempt thereafter to extirpate the intracranial length of the nerve was successful remained in doubt.

The outcome in these younger tadpoles differed markedly from the results of the same operation in the older larvae. Complete recovery was eventually attained in all cases. The

period of paralysis and the early stages of recovery during the first 2½ weeks after operation were not much different from the same in the older animals except that the abnormal wheel movements were less pronounced. Unlike the older cases, however, these animals continued beyond the third week to exhibit gradual improvement in the ocular reflex movements. In the course of 7 weeks during which time the animals grew to a length of about 40 mm the reestablishment of the ocular movements in all 3 planes had become complete.

When the cranium was reopened under anesthesia at the end of 8 weeks, the fourth nerve as well as the third was found to have regenerated and both were quite normal in appearance. Resection of the fourth nerve in 2 of the animals decreased the excursion of the wheel movements only partially showing that the reinnervated inferior oblique muscle had recovered its proper function. Resection of the regenerated third nerve later in the same 2 animals produced the same results as after section of the nerve in normal animals, as described under group I. In 2 cases in which denervation of the lateral rectus muscle was satisfactorily achieved there was a survival of weak movement in the horizontal plane. This, plus the excellence of the recovered horizontal movements and the eye posture in general indicated that the medial rectus muscle along with the vertical recti and the inferior oblique had recovered normal function.

Before concluding that the age difference between these animals and those of group I was the primary cause of the difference in results, a control series of 8 older tadpoles was prepared in which the divided fourth nerve as well as the third was left in a position where it could regenerate readily into its own distal stump. Except that adaptive wheel movements were restored beginning about 5 days after operation the results were very similar to those in group I. It was quite clear therefore that regeneration of the fourth nerve could not have been the factor responsible for the excellence of recovery in group V.

The developmental period during which it is possible to sever the oculomotor nerve and still obtain complete motor recovery is evidently a short one. When the experiments were repeated on an additional 9 tadpoles *(R. grylio)* between 24 and 29 mm in length, the type of recovery in even the smallest of these was much like that observed in group I, even though the animals were kept as long as 4 months during which time they doubled in length.

Apparently, in summary, if the tadpoles are young enough when the oculomotor nerve is divided, there is complete restoration of motor coordination. This is consistent with the type of recovery found by Weiss ('36) to follow the regeneration of limb nerves. It must be remembered, however, that in the oculomotor system this type of recovery is confined to very early larval stages whereas in the limbs it is obtained throughout the larval period and even to some extent after metamorphosis. In what measure the underlying mechanism of adjustment in the 2 cases may be similar remains a problem, for in the oculomotor system there is a complicating factor, namely, the addition of a large percentage of new fibers through rapid growth during the recovery interval.

DISCUSSION

With the exception of group V, the foregoing results resemble more the kind of motor recovery which occurs regularly in mammals than that heretofore found in amphibian larvae. Unlike the limb motorneurons of developing amphibians (Weiss, '36, '41) those of the oculomotor system are apparently able to establish and to maintain functional relations with foreign muscles without undergoing adaptive readjustment in their central timing. This was shown by the failure to recover normal coordination and by the persistence of adverse movements indicative of mass contractions in groups I and IV where there was opportunity for extensive misdirection of nerve fibers. It was perhaps most evident from the establishment and persistence of reversed wheel movements in group II following reinnervation of the inferior oblique mus-

cle by nerve fibers of the superior oblique. A definite distinction must therefore be made between the limb and the oculomotor system with regard to the nature of functional recovery following motor nerve regeneration.

That the mass movements in these cases were due actually to the misdirection of regenerating fibers was evident from the contrasting excellence of motor recovery in group III where conditions otherwise were comparable but fiber misdirection was absent. The traditional assumption that the mass movements which commonly follow regeneration of the facial and oculomotor nerves result from fiber misdirection has been challenged recently by Wartenberg ('46). On the basis of clinical observations he has inferred that they must be due instead to a centripetal spread of the effects of the peripheral trauma which in the centers is presumed to cause a selective breakdown of the more refined, and phylogenetically more recent, coordinating mechanisms and consequently a reversion to a more primitive type of coordination. Unfortunate errors and omissions in the argument as well as contradicting evidence such as the above prohibit any serious consideration of this new hypothesis as it stands.

Only a tendency toward the type of motor readaptation characteristic of limb nerve regeneration in amphibians was seen in groups I, II, and IV in the partial restoration of adaptive coordination of the superior and inferior rectus muscles. Although this recovery of properly directed and properly timed vertical movements was only partially complete, its consistent appearance indicated the existence in the recovery process of some factor favoring systematic reestablishment of correct over incorrect movements. On a chance basis alone, the muscles would have had opportunity after regeneration to be excited as much by antagonist nerve fibers as by their own. Some discriminative agency must therefore have been operative to account for the differential dorsal and ventral deviation of the eye in response each to its appropriate stimulation.

There are 3 general ways possible by which, individually or in combination, these differential responses could be accounted

for: (1) In the reestablishment of peripheral connections formation of proper nerve-muscle relations may somehow have been favored over formation of improper connections. (2) With the structural linkages established, the function of the normal nerve-muscle connections may have been favored over that of the abnormal ones. (3) Motor neurons that had made inappropriate terminations may have undergone adaptive readjustment in the timing of their central discharge. The possibility that the learning process may have been involved may be disregarded. Evidence bearing on this point from many sources is overwhelmingly against it. In the present results the lack of variety in the course of recovery in different animals, the persistence of maladaptive wheel movements, and the survival of the movements after decerebration in 5 cases so tested add confirmation to the conclusion that tadpoles are not capable of learning motor reorganization of this sort. Even in man the incoordinate mass contractions persist indefinitely without correction by reeducation.

It has been inferred that (3) above is primarily responsible for readaptation in the limbs of amphibians (Weiss, '36, '41), but there is little in the present evidence to indicate directly what the correct answer may be for the oculomotor system. Nevertheless, in weighing the various possible explanations it is important to note that all of them demand in one way or another the assumption of motor neuron specificity, i.e. the assumption of some constitutional, perhaps biochemical, property by which the motor neurons belonging to the superior and inferior rectus muscles may have been distinguished from each other.

The complete restoration of normal function in group V was more consistent with the results of nerve regeneration found elsewhere in amphibians. Analysis of the results is complicated, however, by the fact that the animals grew rapidly during the recovery period doubling their length and at the same time adding new fibers to the regenerated oculomotor nerve. Nerve regeneration was thus combined in

these cases with initial outgrowth of new motor axons. It is possible that this addition of new fibers was an important factor in the recovery process along with the relatively plastic embryonic condition of the oculomotor system at this early stage of development.

It is necessary in group V as well as in groups I and IV to assume myotypic specificity of the motor neurons in order to account for the orderliness of recovery. Conceivably the original establishment of central-peripheral relations could have been achieved by orderly topographical and temporal relations in the developing embryo. After the nerve trunk had once been broken, however, leaving a gap in the connections unbridged by any discrete pathways in the intervening substrate, no conceivable method remained by which the functional order could have been restored if it be presumed that the nerve fibers were all alike and remained so. In order that the peripheral and central relations of the individual oculomotor neurons, including the new ones as well as those originally severed, could become mutually adjusted in a systematic adaptive manner there must necessarily have been some means by which the nerves for different muscles could have been distinguished from each other.

Exactly how such neuron specification is acquired and how it becomes effective in regulating the reestablishment of functional relations can only be guessed at present. The explanation suggested by Weiss ('36, '41) to account for the orderly recovery of function following regeneration of limb nerves would be applicable to these results. Accordingly it might be supposed that the muscles impose specific biochemical properties on the axons which grow into them and that this produces a selective sensitization of the nerve cells to particular types of central excitatory agents. The Resonance Principle of central nervous function assumes that the motor neurons are bombarded centrally by excitations for all the muscles supplied from the same nucleus but each responds selectively to only a particular one of the various modes of excitation because of its specific biochemical sensitization.

Organized motor function thus becomes possible on this basis despite the disarrangement of nerve connections in the periphery.

There is an alternative possibility for explaining the recovery of function both in the limb and oculomotor systems which assumes that the rearrangement of peripheral connections in regeneration is followed by a compensatory readjustment in the central connections (Sperry, '41, '43). This scheme involves only an essentially minor modification of that proposed by Weiss but the neurological implications are important because it obviates the necessity for any radical revision of the traditional theory of central nervous integration. The specification of neurons by their end organs — evidence for which has been found in the trigeminal (Weiss, '42), optic (Sperry, '43–'45a; Stone, '44) and vestibular nerves (Sperry, '45b) as well as in the limb and oculomotor nerves — is conceived in this hypothesis to be an important factor both in regeneration and in normal embryonic development, in regulating the formation of central synaptic associations. For example, the nerve cells supplying the superior rectus muscle would acquire synaptic linkages different from those supplying the inferior oblique because of special affinities and incompatibilities between the different motor neuron types and the various kinds of central neurons. These affinities and consequently the pattern of synaptic relations in the oculomotor nucleus, presumably are altered when the nerves regenerate into strange muscles provided that the neurons are still in a sufficiently plastic embryonic condition to permit respecification. The new fibers added in the course of growth could become myotypically differentiated either by the muscles into which they are guided or possibly by more proximal or central contacts with neurons already differentiated. At present either of these explanations must be considered tentative in application to the foregoing results until other possibilities incorporating phenomena under (1) and (2) above have been excluded.

The extent of readaptation after nerve regeneration in the amphibian oculomotor system turns out to be intermediate between that observed in the amphibian limb and that found in mammals. This conforms with the theory that the readjustment process is dependent upon an embryonic plasticity and reversibility of neuron specification. The motor ocular nuclei appear much earlier in vertebrate evolution than the limb centers and in ontogeny they show an earlier and more distinct central differentiation. It would therefore be consistent that they should attain more quickly and completely a state of irreversible specificity. Even the limb motor neurons tend eventually to lose the capacity to shift their myotypic specificity in the adult anuran (Weiss, '36). Whereas in mammals the specificity in the motor neurons of the limb as well as of the oculomotor system presumably becomes irreversibly determined very early in development.

There may be a tendency in the course of evolution for the specification of the nerve centers and the adjustment of their connections to become more and more independent of peripheral regulation wherever possible. If so, this would account further for the difference between amphibians and mammals and between the limb and oculomotor centers in respect to recovery following nerve regeneration. Unlike the motor pools for the limb muscles (Romanes, '41), those for the extrinsic ocular muscles undergo discrete self-differentiation before their axons reach the muscles (Neal, '14), and proper peripheral connections can apparently be preassured to the third, fourth, and sixth cranial nuclei by orderly topographical relations and other factors without need for inductive regulation from the periphery. It is only within the field of the third nerve nucleus itself and its system of peripheral terminations that such regulation would appear to be necessary for economy in development. Conceivably the nucleus might differentiate centrally into its constituent motor pools and thereafter the fibers of each pool might seek out their separate and proper part of the primordial muscle mass in the periphery. It would seem more economical, however, for the nucleus to send

its fibers into the muscle mass indiscriminately allowing specificity of the neurons and their central relations to be induced subsequently through differentiation of the myotome into its separate muscle components.

SUMMARY

1. After intracranial division of the oculomotor nerve in tadpoles and salamanders in mid- and late larval stages, regeneration resulted in persistent mass action of the reinnervated extrinsic ocular muscles indicating that those fibers which had misregenerated into foreign muscles had, in the main, failed to adapt their central reflex relations to suit the new peripheral terminations. Only a tendency toward systematic restoration of adaptive function appeared in the recovery of weak but correctly directed movements of the eye around its anteroposterior axis, dorsally and ventrally each in response to appropriate stimulation.

2. In tadpoles in mid- and late larval stages the branches of the oculomotor nerve were divided separately in the orbit under conditions which allowed the regenerating fibers to restore connections with their original muscles. Under these circumstances regeneration of the oculomotor nerve resulted in an excellent recovery of adaptive motor coordination.

3. In tadpoles of mid- and late larval stages the central end of the divided trochlear nerve was crossed to the peripheral end of the divided oculomotor nerve. The consequent reinnervation of the inferior obique muscle by fibers belonging originally to the superior oblique muscle resulted in wheel movements of the eye in the reverse direction from normal. This provided further evidence that in amphibian larvae oculomotor nerve fibers can establish functional connections with foreign muscles without undergoing adaptive readjustment in their central reflex relations.

4. After intracranial division of the oculomotor nerve in very early larval stages in tadpoles, regeneration was followed by a complete restoration of normal motor function. In this

respect the results in these very young larvae were consistent with those reported by Weiss ('36, '41) to follow regeneration of nerves of the limbs in amphibians.

5. In order to account for the complete recovery of normal muscular coordination in the youngest cases as well as the tendency toward readaptation in the older larvae it is necessary to assume the existence of myotypic specificity among the fibers of the oculomotor nerve. Possibe explanations of the foregoing findings are discussed along with their significance for the problem of the ontogenetic organization of neuronal relations in the vestibulo-ocular reflex arc.

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AMERICAN ASSOCIATION OF ANATOMISTS

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ABSTRACTS

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PAPERS PRESENTED

(The numbers indicate the order in which the papers appear on the program of the meeting.)

40. Origin and activity of primary trophoblast giant cells during implantation (days 4–6) in the albino rat. Roland H. ALDEN, Division of Anatomy, University of Tennessee, College of Medicine.

These cells are believed to be identifiable in the preimplantation stage as enlarged polar (antimesometrial) trophoblast elements, with characteristic hyperchromatic nuclei, at 4½ to 5 days of age. As the embryo becomes situated in the egg-chamber, toward the end of the fifth day, the polar trophoblast cells increase in size and contact the uterine epithelium at the antimesometrial base and sides of the egg-chamber. There follows a very rapid hypertrophy and hyperplasia of the trophoblast elements, not only from the antimesometrial portion of the trophoblast, but from the base of the ectoplacental cone as well. Just where, and only where, primary trophoblast cells contact the maternal epithelium there is evidence

of a disturbance and a subsequent destruction and dissolution of this layer. Careful study of the primary giant cells in normal implantation, investigation of abnormal implantation sites, and employment of artificial ova provide evidence that trophoblast giant cells: (a) are truly of embryonic origin; (b) migrate along the mesometrial-antimesometrial axis of the decidual crypt; (c) phagocytize extravasated blood; and (d) phagocytize uterine epithelial cells.

The frequently mentioned difficulty of distinguishing giant cells from decidual cells, after the disappearance of the uterine epithelium, has been wholly resolved by the employment of an eosin-methylene blue stain, after which the marked and persistent basophilia of the embryonic cells contrasts with the delicate eosinophilia of the decidual tissue.

60. Age changes in the deep cervical lymph nodes of 100 Wistar Institute rats.[1]
Warren ANDREW, Department of Anatomy, Southwestern Medical College.

A series of Wistar Institute rats, ranging in age from 21 days to 1170 days, has been studied. The nodes have been found to present fairly characteristic pictures for the periods which may roughly be called youth, middle-age, and senility. The germinal centers (reaction centers) are just beginning to appear in the 21-day animals. They are large and conspicuous in animals from 50 days up through 300 days and become very definitely reduced in old age, although they are present to some extent in about half of the senile animals (over 700 days of age) and are found even in an animal of 1100 days. In old age the medullary part of the node is much increased at the expense of the compact cortical tissue. Wide sinusoids and even large rounded or ovoid cavities, lined with endothelium, are found in a high proportion of senile rats. Much of the tissue formerly composed of small lymphocytes comes to consist of plasma cells. The capsule of the senile rats frequently is infiltrated with mast-cells and these may enter the node itself.

[1] Part of a program on aging sponsored by Dr. Edmond J. Farris, Executive Director of The Wistar Institute, and supported by the Samuel S. Fels fund. This work was aided by grants from the John and Mary R. Markle Foundation and the Winthrop Chemical Company.

118. Crypt-like analogues in the pharyngeal tonsil. Leslie B. AREY, Department of Anatomy, Northwestern University Medical School.

Descriptions of the pharyngeal tonsil are either erroneous on the subject of crypts by explicit statement, or they fail to indicate the true conditions that exist. The epithelial furrows arise by frank folding and are far more complicated than can be judged from the surface; none is comparable to the crypts of the palatine or lingual tonsils.

Glandular ducts traverse the lymphoid tissue and open either into the furrows or onto the free surface. Many are markedly dilated so that they are tubular, spindle-shaped or conical. Their diameter compares well with the crypts of the adult lingual tonsil and with all but the largest in the palatine tonsil. These expanded ducts are definitely crypt-like, since they extend as diverticula into the substance of the tonsil and increase its epithelial area greatly. Although these

dilatations doubtless function as crypts in the palatine tonsillar sense, they are not homologous structures. Their development is fundamentally that of ducts and they continue normally to serve as outlets from the subjacent glands.

Some blind diverticula occur as well. These are perhaps nothing more than local manifestations of invagination in a region notable for its tendency toward major and minor folding. Much less numerous than the expanded ducts, they conform better to the conventional conception of a tonsillar crypt. It seems probable, however, that their occurrence is incidental.

39. *Studies in gynecologic cytology.* J. E. AYRE and P. CHEVALIER, Gynecytology Laboratory, Royal Victoria Hospital, McGill University. (Introduced by C. P. Martin.)

Using the cervical and vaginal smear techniques, studies have been carried out on the individual cells desquamated from the genital tract. These revealed that normally cyclic changes occurred and that hormone therapy could also induce such changes.

A correlation between the cornified cell count, and the concentration of estrogen in different parts of the genital tract was attempted.

57. *Thymic reaction following injection of colloidal pigments.* Ralph N. BAILLIF, Department of Anatomy, Louisiana State University School of Medicine.

It has been found that after repeated intraperitoneal injections of colloidal pigments (Biebrich's scarlet, mercury sulphide or chlorazol black e) the thymus undergoes involution. In young rats the degree of response is dependent on the amount of pigment injected in a given time; severe reactions appear only when dosage is at the toxic level.

Involution begins with marked destruction of cortical thymocytes and hypertrophy of the reticulum. In this phase there is an accumulation of macrophages and mast cells in the perithymic connective tissue; a few phagocytes are also found in the perivascular medullary reticulum.

The macrophage cells appear to be of 2 functional types. One type, first seen at the cortical periphery, effects pigment granule coalescence with some difficulty, i.e., the ingested globules remain deeply colored, small and discrete. The second type of macrophage, most frequently found in the thymic medulla, is characterized by the presence of large, partially digested pigment enclosures.

Late in the involution process, the lobular cortices become completely freed of their thymocytes. This destruction is carried out by cytolysis following karyorrhexis, or after drawing out the nuclei into tenuous strands. The cortical reticulum is gradually replaced by collagenous fibers interspersed with dye-filled phagocytes and tissue mast cells. During the terminal phase, reticular stroma is confined almost entirely to the perivascular medullary zones. Most of the Hassall's corpuscles undergo complete regression.

81. The effects of small doses of diethylstilbestrol on the anterior hypophysis of thyroidectomized rats. Burton L. BAKER, Department of Anatomy, University of Michigan.

It was demonstrated previously that small doses of diethylstilbestrol administered to immature rats for short periods stimulated the mitotic activity of acidophiles increasing their number and inducing hypertrophy of the Golgi apparatus in these cells. Since these changes are similar to those described in hyperthyroidism, the experiment being reported was designed to test the possibility that estrogen might act on the acidophiles through the thyroid. Forty-four adult female rats were divided into the following groups: ovariectomized, non-treated; ovariectomized, stilbestrol-treated; ovariectomized, thyroidectomized, non-treated; ovariectomized, thyroidectomized, stilbestrol-treated. Six weeks after the operations, 2 μg of stilbestrol were injected daily for 10 days. The hypophyses were fixed in Bouin's fluid and stained with the Masson technique. Differential cell counts revealed: (1) a marked increase in the number of acidophiles in ovariectomized adult rats following estrogen treatment, (2) accelerated mitotic activity did not play as significant a role in this increase as in the immature rat, (3) combined ovariectomy and thyroidectomy almost completely removed the acidophiles, (4) stilbestrol treatment of these animals increased the number of acidophiles to about the level found in the ovariectomized, non-treated controls showing the action of estrogen on the hypophysis to be at least partially independent of the thyroid, and (5) small remnants of thyroid tissue did not affect the response.

65. Some effects of amputation of the chick wing bud on the early differentiation of the associated motor neuroblasts. Donald H. BARRON, The Laboratory of Physiology, Yale University.

Amputation of the wing bud of the chick at circa 72 hours produces no observable effect, in silver impregnated embryos, on the subsequent development and differentiation of the associated segments of the spinal cord until toward the end of the fifth day of incubation when the lateral motor column normally makes its appearance. Thereafter, in the absence of the wing bud, there is a reduction in the number of primary unipolar neuroblasts that become bipolar and lay down the lateral motor column. And apparently as a result of this reduction, fewer indifferent mantle cells differentiate into neuroblasts, for it appears that only those indifferent cells which are in close association with bipolar cells or others more advanced in their differentiation, develop into neuroblasts. As a consequence of the failure of the unipolar neuroblasts and the indifferent cells to complete their differentiation in normal numbers there is a resultant neuron hypoplasia in the lateral motor column as development advances. These results suggest that the axon of a unipolar motor neuroblast by establishing contact with peripheral elements — in this case myoblasts — enables that neuroblast to proceed in its development, and that the bipolar neuroblasts in turn provide a stimulus to indifferent cells which appears to be essential for their differentiation into neuroblasts.

69. *The effect of epinephrin on the pulsation frequency of embryonic heart muscle.* Alexander BARRY, Department of Anatomy, University of Michigan Medical School.

This report covers one phase of an investigation into the development of the physiological properties of muscle. It is concerned with the effect of epinephrin on the rate of pulsation of myocardial tissue of chick embryos between the ages of 40 hours and 7 days. The hearts were excised and placed in aerated, sugar-free Ringer-Locke's Solution at 37.5°C. The entire heart, or specific portions of the heart were mounted in such a way that they could be observed and their pulsations recorded by means of an optical level and slit camera.

When used on slowly beating tissues, it was found that epinephrin in concentrations as low as 1 part in 10 million caused an abrupt acceleration, reaching a maximum rate within a few beats. Usually within 30 seconds this rapid rate began to slow until in 10 to 15 minutes it levelled off. This deceleration was not due to a drop in the concentration of the drug. The degree of this maximal acceleration depended upon the initial rate of pulsation before the addition of epinephrin. The slower the initial rate, the greater was the acceleration; but if the initial rate was faster than 180 beats per minute, the addition of epinephrin produced no significant acceleration. This relationship held true for entire hearts and portions of hearts regardless of the age of the embryo from which the tissue was taken.

95. *Effect of intermittent exposure to 30,000 feet simulated altitude on memory in animals.* R. Frederick BECKER, Department of Anatomy, University of Washington.

Twenty-four young, male guinea pigs were subjected to a simulated altitude of 30,000 feet in a decompression chamber 6 hours daily, 6 days a week for a total of 250 hours. Prior to ascent all animals learned an alternation maze to the point of perfection. Four controls were picked at random from the superior half of initial learners and 5 from the inferior half. All other animals were exposed to decompression. All were retested after 100 hours exposure. No differences in retention of the learned task between controls and experimentals were noted. Groups of 4 experimentals and 2 controls were retested after 150, 200 and 250 hours decompression and sacrificed for study of brain pathology. In all retests controls exhibited perfect retention, while all altitude animals required retraining. The most significant impairment of memory function occurred in the 150- and 250-hour samplings. This impairment occurred without specific correlation with the brain pathology observed.

35. *Ipsilateral conduction in the medial lemniscus of the cat.* C. M. BERRY, R. C. KARL and J. C. HINSEY, Department of Anatomy, Cornell University Medical College.

Cathode-ray records were taken from unipolar electrodes placed in the thalamus and midbrain with the aid of a Horsley-Clarke instrument. Stimulation of both sciatic and both saphenous nerves produced large, positive deviations conducted

at a maximum velocity of 50 m.p.s. when the electrodes were located in the medial lemniscus, in the *pars externa* of the ventral nucleus of the thalamus in the internal capsule. Ipsilateral stimulation produced spikes of about one-half the amplitude of spikes produced by contralateral sciatic and saphenous stimulation.

The exact location of the recording needle in each experiment was determined from serial sections of the brain stem. In the mid-brain, positive responses were localized in an extremely small area on both the ipsilateral and contralateral sides. At the thalamic level, responses were found diffusely in the *pars externa* of the ventral nucleus and in the internal capsule.

22. Phosphatase glycogen relationships in integumentary derivatives. Gerrit BEVELANDER and P. L. JOHNSON, Department of Anatomy, New York University, College of Dentistry.

In several previous studies we have shown that alkaline phosphatase and glycogen are present in the developing tooth and hair; we also pointed out a number of correlations in regard to growth, differentiation and spacial relationships of glycogen and phosphatase during development. We have now extended our observations to include a study of the interrelationships between phosphatase and glycogen in the developing feather. In this latter structure we observed that phosphatase is intimately associated with the mesodermal components of the developing feather, particularly during the period of active growth and differentiation. Glycogen makes its appearance on the thirteenth day of development. It is confined to the barb cells and by the seventeenth day it disappears completely. The similarities and differences of the phosphatase glycogen relationships of these integumentary derivatives together with certain aspects of functional significance which these substances may play in development will be discussed.

2. Experimental implantation in the rat and guinea pig. Richard J. BLANDAU, Department of Anatomy, University of Rochester, School of Medicine and Dentistry.

The current concepts of the mechanism of implantation include 2 opposite views regarding the specific role of the ovum in the implantation process. One group of investigators considers the blastocyst an active agent facing a passive endometrium, whereas another group holds that the ovum acts as an inert object and that the reactions of the endometrium are largely responsible for implantation. In order to obtain further information on this problem, glass or paraffin beads of the approximate size of the blastocyst, were inserted in the uterine lumina of rats and guinea pigs between the second and sixth day after the onset of behavioral estrus. The animals were subsequently killed between the eighth and twelfth days after the onset of heat and their uteri were examined to determine the fate of the beads. A definite species difference was observed in the endometrial response. In the rat the formation of the decidual crypts and the implantation of the beads into them occurred in the same manner and rate as did implantations of ova in the controls. In the guinea pigs, on the other hand, the beads did not penetrate the uterine epithelium and the decidual reactions induced by their presence in the uterine lumina were minimal.

66. *Growth of the lumbosacral nervous system of chick embryos after the substitution of tumors for the limb periphery.* Elmer D. BUEKER and Morris BELKIN, Departments of Anatomy and Pharmacology, Medical College of the State of South Carolina.

Hind limb primordia were removed from the right side of the 48–60-hour embryonic chick. Small pieces of Rous fowl sarcoma, mouse sarcoma 37 and 180, and mouse adenocarcinoma were then fitted into the limb region. After a total of 8–9 days incubation animals were fixed in Bouin's fluid, imbedded, sectioned at 10 μ and stained with hematoxylin. Studies were then made of the right and left sides of the lumbosacral nervous system.

Results of 50 experiments were as follows: (1) mouse adenocarcinoma transplants are mostly absorbed and the growth pattern of the lumbosacral nervous system at 8–9 days total incubation was similar to cases of radical limb extirpation; (2) mouse sarcoma 37 and 180 and Rous fowl sarcoma develop tumor masses at the limb site; (3) in these cases the lumbosacral nerves are well developed and spread out irregularly in the tumors, cells of the lateral motor column are reduced 30–60% while spinal ganglion cells approach the normal number or become hyperplastic. General results indicate that a sarcoma may be an adequate environment for the differentiation of spinal ganglia.

7. *Studies on the sebum on the forehead.* E. O. BUTCHER and J. P. PARNELL, Department of Anatomy, New York University.

Sebum was collected at the hairline weekly and daily from several individuals. Associated with an oily scalp was the most fat and the lowest percentage of cholesterol. Associated with scalps having dandruff was less fat and a greater percentage of cholesterol.

The amount of sebum which could be collected weekly or daily at the hairline from the individuals varied considerably. Much more sebum could be collected from an area which was covered by a shallow cup for several hours than from the same area after it had been uncovered for a similar interval.

Investigations showed that the decreased amount from the uncovered area was not due to votalization of the sebum, that the increased accumulation on the covered area was not due to the stimulation of the moisture under the cup, but it was due to the increased temperature resulting from the covering.

54. *The cell lineage of the inflammatory cells of acute encephalitis with a discussion of the significance of the plasma cell.* Berry CAMPBELL, Department of Anatomy, University of Minnesota Medical School.

A study was made of the development of the inflammatory cells of the early stages of herpetic encephalitis in rabbits. The formation of the perivascular cuff with the differentiation of infiltrating lymphocytes to form polyblasts and finally the macrophages has best been understood through the combined study of convential H. and E. stains and air dried Romanowsky-stained imprints. In early encephalitis there is differentiation of some of the polyblasts to form plasma cells. This cytomorphic sequence is of importance in that it links the earliest stages of this disease to allergic processes. Reference to other experimental work supporting this interpretation will be made.

Progressive changes in both the oligodendroglia and the microglia occur in the early stages of encephalitis. These consist of an "activation" of the cells resulting in the formation of a larger stainable cytoplasm, a rounding up of the cell, an increase in the chromatin content of the nucleus, and thickening of the nuclear membrane. A close similarity of the oligodendroglia to the inactive reticulo-endothelial cells of the connective tissue (the resting wandering cells) is suggested by these findings.

111. *Effects of use and disuse on neurosomes in skeletal muscle of the chameleon.*[1] Eben J. CAREY, Leo C. MASSOPUST, Walter ZEIT, and Eugene HAUSHALTER, Department of Anatomy, Marquette University School of Medicine.

The effects of unilateral tenotomy and immobilization by adhesive tape casts were observed on the neuromuscular apparatus of the control and experimental gastrocnemius muscles of the chameleon. The normally used and innervated gastrocnemius muscle has dark, granular, and light, agranular muscle fibers. The dark, granular muscle fibers progressively disappeared during the atrophy of disuse. The altered nerve ending during atrophy discharged small and giant fusiform neurosomes. The giant neurosomes were the product of retardation in rate of discharge, diffusion, and disappearance by hydrolysis during the atrophy of disuse. The experimental evidence suggested that one factor in the atrophy of muscle by disuse was a substantial loss of the normal discharge of neurosomes from the nerve endings in skeletal muscle.

[1] Aided by a grant from the National Foundation for Infantile Paralysis, Inc., and the Baruch Committee on Physical Medicine.

62. *Some preliminary observations on the descent of the testis in man.* Simon B. CHANDLER, Department of Anatomy, West Virginia University School of Medicine.

The differential growth between the trunk and the gonad is said to bring about a caudal shift of 10 segments in the position of the testis. Does the gubernaculum cause further descent by active as well as relative shortening? Moscowicz concluded that the gubernaculum is composed of mucoid connective tissue which first swells up to dilate the inguinal canal and then loses its sustaining power so that the testis sinks downward by a normal herniation process carrying the processus vaginalis with it.

This report is based on dissection of the inguino-hypogastric region in 21 fetuses whose crown-rump length varied between 9.5 cm and 23.5 cm. In fetuses from 9 to 10 cm long the processus vaginalis was about 1 mm in length. In those 17 to 18 cm long the testes were at or near the internal orifice of the processus, and inside the canal in those near 23 cm long.

Grossly the gubernaculum resembles umbilical cord. Histologically it is apparently mucoid connective tissue. In no specimen was it as large as the testis so it probably does not dilate the canal. Since it is small the testis probably could not sink down into its substance nor herniate the processus vaginalis.

13. The architecture of the Achilles tendon. K. S. CHOUKÉ, Department of Anatomy, Graduate School of Medicine, University of Pennsylvania.

The architecture of tendons has not received as much consideration as that of bones and muscles. This may be due to the difficulty of working out the structural patterns in tendons and in part to the lack of appreciation of their value. The tendon fibers from the lateral head of the gastrocnemius insert in front of the tendon fibers from the medial head. This slight torsion has been diagramed as an exaggerated helix. The anterior-posterior arrangement in the human has been studied by White who made use of it in devising a technic for tenotomy. The textbooks and handbooks of anatomy do not describe this torsion.

In 62 cadavera the arrangement of the tendon into 2 distinct, separable parts was found bilaterally in every instance; generally the tendon from the lateral head was anterior to the medial head tendon. In one cadaver the tendon relations were reversed bilaterally. This reversed condition was found in 3 other cases; 1 on the left side and 2 on the right. The operative technic recommended by White for tenotomy would work equally well in these variants. This reversal would rule out a correlation of this twist in the tendon with the torsion of the limb in development. A ready explanation for the occurrence of this pattern or its reversal is not evident.

112. The "interstitial cell net" of Cajal in the skin of mouse. C. H. U. CHU, Department of Anatomy, Washington University and The Barnard Free Skin and Cancer Hospital, St. Louis. (Introduced by E. V. Cowdry.)

Two kinds of nerve elements are observed in mouse skin fixed in formic acid and stained with hematoxylin. The one conforms with the interstitial cell net first described by Cajal (1889); the other appears as subcutaneous and subepithelial plexuses.

The interstitial cells (Cajal) vary from fusiform to multipolar according to the number of processes, which, often varicose and tortuous, anastomose with those of neighboring cells at different levels forming a network throughout the entire thickness of the dermis. Where there are few processes, the cell resembles a Remak's knot. At bifurcations, a fibroblast is often lodged. The interstitial cells differ from Schwann cells in that the width of the nucleus of the former often exceeds that of its processes.

The question whether the interstitial cell net is intrinsic has a significant bearing on functional interpretation. The spinal nerve forms a myelinated subcutaneous plexus which continues peripherally as an amyelinated subepithelial plexus. The subepithelial plexus intertwines repeatedly with the interstitial cell net, yet no continuity has been observed. The pseudopodial extension of fibroblasts at bifurcations might account for a different interpretation. Further observation reveals that the spinal nerves also possess an interstitial cell net which is seen to be continuous with that in the dermis.

This study leads to the belief that the interstitial cell net is of extrinsic origin and that the cells are lemmocytes.

6. *Arterial anastomoses — some factors concerned in their formation.* Eliot R. CLARK and Eleanor Linton CLARK, Department of Anatomy, University of Pennsylvania.

The great variation in arterial anastomoses in different parts of the body — a matter of great clinical importance — stimulates the question: why?

The governing factor appears to be Thoma's histomechanical principle, that the size of the lumen of an artery is regulated by the amount of blood flow. In the absence of flow, the lumen is reduced to zero and the artery obliterated. In order that anastomoses may survive, conditions must be such as to provide a flow of blood through the final connecting part.

Observation of living vessels both preformed and newly-formed in the rabbit's ear, as seen microscopically in artificially installed chambers, reveals, as an immediate factor for maintenance of arterial anastomoses, frequent reversal of blood flow in the terminal connecting portions. This is caused by unsynchronized contractions of arteries or arterioles.

This internal vasoformative factor must be added to the factor of varying external pressures that undoubtedly operates in causing reversals of blood flow in the exposed parts of the body.

If correct, one could predict that organs with very small or no arterial anastomoses, that are protected from varying outside pressures, will show little if any unsynchronized arterial contraction. This has been borne out in observations of the cerebral circulation (Wentzler); and of certain ear chambers in which arteries failed to receive a nerve supply, and were therefore without nerve-controlled contractions.

29. *Visual disturbances following frontal ablations in the monkey.* George CLARK and K. S. LASHLEY, Yerkes Laboratories of Primate Biology, Orange Park, Florida.

Confirming the report of Kennard and Ectors ('38) and of Kennard ('39) it has been possible to produce an homonymous hemianopsia by ablations of portions of the frontal lobes. The destruction must be more extensive than indicated in the original reports. In addition to the area within the arcuate sulcus much of the tissue mediad and rostrad must be removed. It was also possible to produce an homonymous hemianopsia by a transverse lesion of the medullary substance of the cerebrum severing the superior longitudinal fasciculus. Deviation of the head and eyes and circling do not necessarily accompany the visual defect. The duration of the visual defect is correlated with the amount of destruction. Eye movements in all directions in response to visual stimulation in the intact field (ipsilateral to lesion) are normal as are movements in response to auditory stimulation. It is suggested that the visual defect represents a traumatic disorganization of reentry circuits producing interaction between the frontal and occipital regions.

108. *Gross structure of the supra-pubic and pre-pubic subcutaneous layer in the male.* E. D. CONGDON, Department of Anatomy, Chicago Medical School.

Photographic records of serial sections in 27 male bodies show no superficial fascia passing down in front of the pubis. Instead of this, a rather complex and

specialized system of fibrous structures was found. They include a tract in the upper pre-pubic region lying in the mid-plane of the body and dividing to form a loop around the free part of the penis. It corresponds roughly to the fundiform ligament of the penis as described by authors from dissections. This ligament is clearly distinguished from the suspensory ligament of the penis.

Traced cranially, the median tract is seen to break up into laminae in the supra-pubic region. These laminae, taken with the masses of fatty areolar tissue they enclose, form, when well developed, a clearly delimited body of pattern different from that of the panniculus adiposus lying superficial to it. This body tapers cranially and soon terminates.

The structures described show connections not only with the penis but with the scrotum. Fibrous condensations partly invest the spermatic cords in the pre-pubic region. They connect, toward the mid-line, with the median tract and they go over on either side distally into the superficial fascia of the corresponding half of the scrotum. Also in the mid-line, the fascia in the scrotal septum may be attached to the loop.

83. Effects of hypophysectomy upon gastric acidity of adult female rats. Roger C. CRAFTS and Burnham S. WALKER, Departments of Anatomy and Biochemistry, Boston University.

Hypophysectomy produces a microcytic hypochromic anemia in the rat. Injections of thyroxine, iron, and copper alleviate this anemia. In a study of the possible effects of hypophysectomy on iron metabolism, gastric acidity was the first aspect investigated.

Adult female rats of the Long-Evans strain and of 3 to 4 months of age were divided into 3 groups, (1) 11 normal control rats, (2) 12 hypophysectomized rats, and (3) 10 rats bled from the heart until anemic. After anemia had developed in the last 2 groups of rats, stomach contents were studied immediately after anesthetization with ether (gastric sample no. 1), ½ hour after anesthesia (gastric sample no. 2), ½ hour after an injection of 0.04 mg of mecholyl per kilo body weight (gastric sample no. 3), and 1 hour after the above injection (gastric sample no. 4).

Determinations were made on pH, total acid, free acid, hemoglobin, and total erythrocyte count. Erythrocyte counts and hemoglobin values for the normal control rats were 8.40 million cells per mm^3 and 15.1 gm per 100 cm^3; for the hypophysectomized rats, 5.60 million and 9.3 gm; for the bled rats, 5.42 million and 9.1 gm. In all samples the hypophysectomized rats had lower gastric acidities than the control rats. The bled rats had normal or elevated gastric acidities. Free acid was found in 8 samples from control rats, in 2 samples from hypophysectomized rats, and in 14 samples from the bled rats.

Hypophysectomy decreased gastric acidity in adult female rats. This decrease was not due to the anemia which was induced by hypophysectomy.

104. Genetic differences in the ossification pattern of the rabbit. D. D. CRARY and P. B. SAWIN, Department of Biology, Brown University.

The possible effect of genetic and environmental influences upon the pattern of first appearance of ossification is at present controversial, largely due to in-

adequate genetic control of the material previously used. A series of roentgenograms of 101 rabbits of 2 inbred races (III and X) has been made during the first 20 days post-partum, during which time most of the epiphyseal centers of the limbs are established. Forty of the race X individuals are heterozygous for the dwarf gene (dw). The order of first appearance of the centers in the 3 different populations is essentially the same except in carpals and tarsals, but time of appearance varies between the 2 races, the larger initiating ossification the earlier. In spite of a significant difference in body weight between the homozygous and heterozygous populations of race X, there seems to be little significant difference in the time of first appearance of the centers. Thus whereas the specific gene (dw) appears to have no influence upon the time of appearance of centers, the genes determining the racial differences in body size do seem to have a specific effect. Since it has been previously shown that ossification of the ribs occurs earlier and more anteriorly in race X than in race III, it becomes apparent that the specific genes controlling rib ossification are different from those controlling limb ossification.

101. Anomalous right subclavian artery originating from the descending aorta. Arthur DALTON, Resident in Surgery, St. Louis City Hospital, and William F. ALEXANDER, Department of Anatomy, St. Louis University School of Medicine.

The right subclavian artery arises from the right side of the descending aorta at the level of the lower border of the fourth thoracic vertebra. It crosses the vertebral column transversely, partially separated from the posterior surface of the esophagus by the thoracic duct.

At the right side of the fourth thoracic vertebra it executes a right angle and ascends along the lateral aspects of the vertebrae paralleling the esophagus, but on a more posterior plane. As it turns lateralwards to leave the thorax it lies directly anterior to the first thoracic sympathetic ganglion.

Other relations were essentially normal except the origin of the right common carotid which arose from the right part of the aortic arch.

This variation of the origin of the right subclavian artery is the first encountered in observations on 193 cadavers, a percentage of .52. Its topography is such that it falls within Piersols' Group II of these variations.

115. Histogenesis of the argentophile cells of the proventriculus and gizzard of the chicken. Alden B. DAWSON and Sue L. MOYER, The Biological Laboratories, Harvard University and Radcliffe College.

These cells are demonstrable by the Bodian protargol method (even without reduction or gold toning) and the Gros-Schultze method, but are not chromaffin positive and are not impregnated by the Masson-Hamperl technique. Accordingly they are referred to as argentophile rather than argentaffin. In contrast, the majority of morphologically similar cells in the intestine are argentaffin. Argentophile cells first appear in the superficial epithelium of the proventriculus on the eighth day of incubation when the compound glands are seen as simple sacs, and reach their maximum concentration by the thirteenth day. Their distribution is

modified by the development of superficial plicae, apparent by the sixteenth day, and they are, thereafter, progressively reduced in number. Argentophile cells of the compound glands develop in situ and are present in the primary glandular sacculations by the twelfth day. They increase in number rapidly as each saccule becomes multilobed and acquires secondary evaginations which eventually become tubular glands. Argentophile cells become most numerous on the periphery of the compound glands and at first are rounded. Shortly after hatching they become greatly elongated, lie scattered over the surfaces of the compound glands or are radially arranged between the distal ends of the constituent tubules. Argentophile cells appear in the epithelium of the gizzard 3 or 4 days before hatching. They never become numerous and always remain rounded in form.

129. Changes in the adrenal cortex and thymus induced by different B-vitamin deficiencies. Helen Wendler DEANE, Department of Anatomy, Harvard Medical School, and James H. SHAW, Harvard School of Dental Medicine.

Dietary deficiencies of thiamine, riboflavin, and pyridoxine were produced in young male rats. With riboflavin and pyridoxine deficiencies growth continued, although slowly, throughout a 10 weeks' period. With thiamine deficiency growth ceased after 2 weeks, and loss of weight ensued until death at 4 to 5 weeks. All 3 groups showed inanition due to loss of appetite. Of 2 control groups on a normal diet, one was fed ad libitum, the other pair fed with the thiamine deficient animals.

With thiamine deficiency the adrenals hypertrophied, while the thymus atrophied. Cytochemical preparations showed that the ketosteroids (sudanophilic, Schiff-positive, birefringent, and fluorescent lipids) of the zona fasciculata of the adrenal cortex increased in quantity by 2 weeks and then disappeared by 4 weeks. The mitochondria became swollen and difficult to stain. This stimulation, followed by exhaustion, of the fasciculata is attributed to a high level of secretion of adrenotropin under stress. Inanition controls experienced less atrophy of the thymus and slower stimulation of the adrenal cortex; consequently the deficiency changes were not the result of inanition alone.

With the absence of riboflavin or pyridoxine the adrenals hypertrophied only temporarily (probably a result of mild inanition), and no marked cytochemical alteration was observed. With pyridoxine deficiency, however, the thymus atrophied rapidly, presumably independently of adrenal stimulation.

1. Electromyograms of wrist muscles correlated with wrist statics. Wilfrid T. DEMPSTER, Department of Anatomy, University of Michigan, and John C. FINERTY, Department of Anatomy, Washington University.

In holding and supporting weights with the hand and forearm horizontal, various combinations of adjacent forearm muscles must resist the pull of gravity. Neither anatomical nor mechanical analyses tell how the various muscles share in the task. An action potential study was made of various (10) regions of the forearm muscle mass using surface electrodes over specific muscles. Motor points served as loci for the proximal electrode. Curves showing potentials correlated with the torque due to the weight of the empty hand and torques to 32x or 64x show increasing potentials with increased strength of isometric contractions.

Rotation of the hand and forearm through 12 stations 30° apart permitted potentials to be recorded for different directions of gravitational pull. Agonist, antagonist and lateral stabilizing actions of 10 muscles (?) could be compared. Although potentials of adjacent muscles are sometimes superimposed in one direction or another, a basic pattern of activity appears. Typically, the activity of a muscle is maximal when that muscle is in the direct agonist position and it decreases as the direct supporting function changes toward lateral stabilization. Action at a right angle to the pull of gravity may be as low as half the maximal agonist strength. As a muscle again approaches the vertical, lateral stabilization gives way to antagonist activity and this may be as low as a third of maximal agonist values for the weight used.

52. The development of plasma cells in the organs of animals receiving various antigens. Thomas F. DOUGHERTY and Harald GORMSEN, Department of Anatomy, Yale University and Department of Pathology, University Institute of Forensic Medicine, Copenhagen.

Single injections of antigen (staphylococcus toxin) resulted in an increase in the numbers of highly basophilic lymphoid cells in the spleen and lymph nodes of treated mice. These cells have been identified by various authors as immature plasma cells, reticular lymphocytes or acute splenic tumor cells. These immature cells are capable of differentiating to typical plasma cells and lymphocytes.

Prolonged treatment of rabbits with polyvalent pneumococcus or salmonella vaccines and of mice with either sheep cells or staphylococcus toxin also resulted in intensive proliferation of the immature basophilic cells in the lymphoid tissues. The number of typical mature plasma cells in the lymphoid tissues was far greater in the animals that received successive dosages of antigens.

Formation of immature basophilic lymphoid cells or of mature plasma cells was not observed in the thymi of animals receiving single or successive doses of antigen.

Plasmacytic reticular cells in the bone marrow increased markedly following injection of various antigens and appeared to differentiate to typical bone marrow plasma cells.

Extracts of non-lymphoid tissues of immunized animals containing numerous plasma cells yielded large amounts of antibody. In this respect plasma cells are similar to lymphocytes. The progressive increase in numbers of immature, basophilic lymphoid cells and mature plasma cells following successive doses of antigens is correlated with the development of the hyper-immune state.

53. Fibrous connective tissue of the rabbit ovary. Kenneth L. DUKE, Department of Anatomy, Duke University School of Medicine.

Differential stains for reticular, collagenous and elastic fibers were used on sections of rabbit ovaries covering the period from sex differentiation to maturity.

Reticular fibers appear first and are clearly seen in 22-day embryos. The fibers, in the mesovarium, extend into the epithelial core, insinuating between groups of cells. The ingrowing sex-cords are partially encased with a reticular capsule and the anlagen of the tunica albuginea is visible. The medullary fibers increase at the expense of the epithelial constituents, and radiating trabeculae increase in

number and size as development proceeds. The theca interna is invaded by reticular fibers and interstitial cells are enmeshed in a reticular network.

Collagenous fibers show clearly in 27-day embryos. The distribution is similar to the reticular fibers but not so extensive. In older stages collagenous fibers are most pronounced in the tunica albuginea and the medulla.

Elastic fibers do not appear in the ovary until 4 days after birth when the proximal part of the ovarian artery develops an internal elastic membrane. An elastic interna in veins and externa in arteries does not develop until later: about 20 days after birth. Not until shortly before maturity do the distal portions of the vessels exhibit elastic fibers. Ovaries containing masses of interstitial cells and/or regressing corpora lutea show arteries with a laminated external elastic membrane.

103. The femoral venous system. Edward A. EDWARDS, Department of Anatomy, Harvard Medical School.

The femoral venous system was examined in 60 limbs, with reference to its operative interruption.

The *deep femoral vein* was constituted by the confluence of the perforating veins. It entered the femoral vein at an average of 8 cm below the inguinal ligament. It communicated with the femoral just above the adductor opening in several instances.

The *medial and lateral femoral circumflex veins* ordinarily terminated independently in the femoral above the deep femoral termination. The medial was the higher, entering at the same level as the saphenous, or above.

Collaterals of the femoral veins were often seen below the level of the deep femoral termination. *Anastomoses* between the femoral and pelvic veins consisted of 2 pathways: a medial, between the medial femoral circumflex, and the obturator, and inferior gluteal veins: and a lateral, consisting of an anastomotic circle from the lateral femoral circumflex vein, or the femoral above this level, to the deep circumflex iliac and external iliac veins. The valves of these anastomotic routes have been noted in this study.

Obstruction to venous return is least when the femoral is interrupted below the deep femoral termination. The anastomotic routes are small and devious when the femoral is interrupted above the deep femoral but below the circumflex femoral veins. The pathways described, constitute large and direct anastomoses, when the interruption is done above the circumflex femoral veins. The limitations imposed by their valves disappear when the gradual venous dilatation brings about a valvular insufficiency.

20. The effect of testosterone propionate on the alkaline phosphatase of the adrenal cortex of the mouse. Herbert ELFTMAN, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Alkaline phosphatase is present in marked concentration in both nuclei and cytoplasm of the adrenal cortex of the adult male mouse. In the adult female this enzyme is present only in traces in the adrenal cortex. The distribution characteristic of the adult male appears during post-natal development concurrently with

the regression of the X-zone. The dependence of this distribution on androgen is indicated by its disappearance in the adult male after castration and its appearance in castrated males and females and in juvenile males and females after the administration of testosterone propionate.

116. Visualization of histological structure: The liver. Hans ELIAS, U. S. Public Health Service.

The teaching of histology is perhaps the most difficult part of medical education because the approach to this subject differs from that to all subjects with which students have previously come in contact.

Histological structure is a 3 dimensional affair. But it is customary to present it in the form of 2 dimensional slices. Much experience and a special stereographical talent are required to visualize the complicated relationships of the elements of an organ.

Modern histology teaching ought to be physiologically and embryologically oriented. The histology teacher ought not to spend all his time on the explanation of spatial relationships, but he ought to use as much of his effort as possible to explain the reasons for the existing structure.

In order to free teachers from lengthy discussions of structure, a series of slide films is being created to provide a stereographic synthesis of the architecture of organs. The first unit chosen is the liver because, according to the writer's experience, this is one of the most difficult chapters of a histology course, for students as well as for teachers.

44. Regenerative processes induced by gonadotrophic hormones and pituitary implants in x-ray damaged ovaries of mice. J. M. ESSENBERG, Department of Anatomy, The Chicago Medical School.

The ovaries of mice seem to be more easily damaged by x-ray than those of other mammals. This experiment was designed to test the regenerative power of damaged ovaries of young mice by follicle-stimulating hormones.

Four litters of young mice of ages 3 to 4 weeks were given 244 to 300 Roentgen units of x-rays. About one-third of these were set aside as treated controls. The remaining mice were divided into 2 groups; one to receive injection of gonadophysin (G. D. Searle and Company) and the other group for pituitary implant. From 10–15 R.U. of gonadophysin was given to each mouse of the first group twice weekly for 3 months; the mice of the second group received pituitary implant weekly for 3 months. During the 3 months samples were taken from all groups for study. At the end of the 3 months the remaining females were mated to normal males.

With the dosages used the ovaries are seriously damaged and remain so in most cases for life. The gonadophysin treated ovaries show active germinal epithelium and the formation of young follicles. The ova of these follicles soon atrophy. The same is true of the ovaries of pituitary implanted mice but less consistently.

It is concluded that in the albino mouse definitive germ cells arise from the germinal epithelium.

12. The behavior of the femur as an elastic body. F. Gaynor EVANS and H. R. LISSNER, Departments of Anatomy and Engineering Mechanics, Wayne University.

The use of the "stresscoat" technique provides a new approach applicable to both static and dynamic studies of the problems of bone and skeleton architecture and mechanics. "Stresscoat" is the trade name for a brittle lacquer which responds to tension deformation occurring in the object upon which the lacquer is sprayed. The lacquer is applied under known temperature-humidity conditions. The lacquered femur is placed in a materials-testing machine, and during the test cracks appear in the lacquer where fracture would eventually occur.

In the present tests cracks appear almost simultaneously on the superior surface of the neck and the lateral surface of the shaft about 6 inches distal to the gluteus minimus insertion. When a lacquer with a sensitivity of 0.0018 inches per inch was used cracks first appeared when the load reached 652.5 pounds (average). The nearer the load and the anatomical axes coincide the greater the load borne before cracks appear in the lacquer. Femurs become noticeably bent during the tests but quickly return to normal after removal of the load.

The method of determining the various reference points on the femur and the orientation of the bone in the testing machine are illustrated.

85. Gigantism produced in normal female rats by chronic treatment with pure pituitary growth hormone. II. Skeletal changes. H. M. EVANS, H. BECKS, C. W. ASLING, and C. H. LI, Institute of Experimental Biology and Division of Dental Medicine, University of California.

Gigantism was induced in 8 normal female rats by daily injection of pituitary growth hormone for 437 days, after their growth curves had plateaued. Their skeletons were examined roentgenographically and histologically, and compared with those of 5 normal controls.

Linear increase in the bones measured was proportional to that of the entire body. The average length of tibias was 44.6 mm for injected rats, and 39.7 mm for controls. Caudal vertebrae increased in length, the ninth segment measuring 10.6 mm in injected rats and 9.4 mm in controls.

Metacarpal and metatarsal maturation had occurred before onset of injections, and overgrowth of paws was not seen.

Endochondral ossification was studied at the proximal end of the tibia, at the costochondral junction and in caudal vertebrae. Injected rats had an average tibial epiphyseal cartilage width of 154 μ, compared with 126 μ in controls. In all epiphyses examined there was persistence of some zonal arrangement in the cartilage, erosion of cartilage at point of contact with capillary tufts, and formation of delicate trabecular bone, indicating continuing chondrogenesis and osteogenesis. Controls showed atrophic cartilage subject to little or no erosion; capillary tufts were rare; bone trabeculae were coarse and few in number.

Intramembranous ossification was stimulated, as shown by lines of accretion at the sagittal suture as well as on inner and outer tables of parietal bones.

19. *Factors influencing accumulation of histochemically detectable cholesterol in the corpus luteum of the rat.* John W. EVERETT, Department of Anatomy, Duke University School of Medicine.

In the rat corpus luteum widespread accumulation of cholesterol, demonstrable by the Schultz reaction, apparently can occur only after very recent luteotrophic stimulation. In the presence of the hypophysis, cholesterol deposition in pregnancy corpora lutea has regularly followed injection of estradiol benzoate in oil (2 μg or more) and has occurred approximately coincidentally with induced ovulation (ca. 36 hours after injection on the fourth day of pregnancy in our standard procedure). The identical chronological pattern of events was caused by minute estradiol crystals implanted at a similar time, provided that they remained in the animals longer than 24 hours. LH injected on the fifth day of pregnancy regularly caused both ovulation and cholesterol deposition within 18 hours, even when the hypophysis was removed at the time of injection. However, if the gland had been excised earlier (24 hours or more) neither event transpired. In hypophysectomized rats in which corpora lutea were maintained by LH-free lactogen, injection of LH induced cholesterol deposition, while administration of estradiol benzoate failed.

Estrogen probably brings about this accumulation of cholesterol by liberating large amounts of hypophyseal LH. In some manner LH affects actively secreting luteal cells to deposit cholesterol therein. If this latter substance is a natural precursor of progesterone as certain evidence implies, its accumulation suggests interference with, or possibly undue acceleration of, early steps in elaboration of corpus luteum hormone.

77. *Autoplastic and homoplastic transplantation of rat adrenal components to the kidney.* Newton B. EVERETT, Department of Anatomy, University of Washington.

In an attempt to determine the capacity of adrenal components to become functional in a heterotopic position transplants of the adrenal capsule, and of combined glomerular and fascicular zones were made to the kidney of the same animal, to litter mates and to less closely related animals. Pieces of the medulla were autotransplanted. One adrenal was removed from each of the hosts receiving an autoplastic graft. All transplants remained on the host for a minimum of 3 weeks.

The cortical transplants to non-related hosts were negative, 25% of those to littermate hosts were positive, and 75% of the autotransplants were positive. Both capsular, and combined glomerular and fascicular tissue were incorporated as successful grafts. These increased in size, showed mitoses, and the cells appeared physiologically active.

Thirty-three per cent of the autoplastic medullary transplants were positive in that the tissue persisted and was recognized as typical medullary tissue, but there were no indications of an increase in the component parts.

17. *Anatomical studies on jet penetration of human skin for subcutaneous medication without the use of needles.* Frank H. J. FIGGE and Robert P. SCHERER, Department of Anatomy, University of Maryland School of Medicine.

When it became known that the human skin could be penetrated by high velocity fine bore oil jets from Diesel engines, one of us (R.P.S.) designed and perfected an instrument to utilize this principle for subcutaneous administration of drugs without the use of needles. The present paper will describe the investigations on the anatomical potentialities of this instrument. A number of factors were found to influence the practical and physical aspects of skin penetrability. The velocity of the jet could be regulated by the instrument spring tension. The diameter of the jet could be regulated by the size of the opening in the ampule pressure chamber. By using jets of very small diameters it was found that materials could be injected subcutaneously with far less pain than is elicited by the introduction of a needle.

A variety of substances were injected into fresh unembalmed cadaver extremities and other parts of the body to determine depth of penetration, deposition pattern, and the practical limitation of the method. The instrument injected aqueous solutions, oils, colloidal suspensions, emulsions, and even mercury. The material injected with a jet was deposited over a wider area than that injected with a needle. Skin tension was found to be a more important factor in regulating penetrability than skin thickness. Kodachrome pictures will be projected to facilitate presentation of this data.

48. *A method for the bio-assay of minute amounts of progestin.*[1] Thomas R. FORBES and Charles W. HOOKER, Department of Anatomy, Yale University.

A method has been devised for application to unconcentrated biological materials which involves intra-uterine injection of mice. By micrometer advancement of the plunger of a syringe, 0.0006 ml of material is injected into a 5 mm uterine segment, defined by ligatures, in a young adult mouse ovariectomized 16 days earlier. A shorter period after ovariectomy decreases the uniformity of the response. The test animals are killed 48 hours after injection; shorter intervals between injection and autopsy decrease the sensitivity of response. The injected segment is fixed in Lavdowsky's fluid; 6 micron paraffin sections are stained with hematoxylin. A positive response to progestin consists of transformation of dense, fusiform stromal nuclei into enlarged, vesicular nuclei with fine chromatin particles and distinct nucleoli.

Under these conditions the minimum effective dose of progesterone dissolved in sesame oil has consistently been 0.0002 μg. The response has not been evoked by intrauterine injections of estradiol, testosterone, desoxycorticosterone acetate, or serum from ovariectomized mice, and it was not modified by estradiol given simultaneously with progesterone. A crude extract of sows' corpora lutea, liquor folliculi of sows, and serum from ovariectomized mice given 2 mg of progesterone 6 to 7 hours earlier each provoked a positive response.

[1] Aided by grants from the Committee for Research in Problems of Sex, National Research Council, and from the James Hudson Brown Memorial Fund of the Yale University School of Medicine.

88. Spike potentials in sensory fibers from the knee joint of the cat. Ernest GARDNER, Department of Anatomy, Wayne University.

It has been shown that some myelinated fibers in an articular twig of the posterior tibial nerve are derived from Ruffini-type endings in the posterior part of the capsule, while others accompany blood vessels to other regions of the knee joint. In the initial study of functions of these fibers, 3 different methods were used. (1) The sciatic was cut and all distal branches except the posterior tibial and its articular branch severed. When the sciatic was stimulated, no muscle contractions resulted. The spike recorded from the articular nerve following such stimulation was the compound potential of all A fibers in the nerve. (2) Spikes were recorded after stimulation of dorsal roots. The inflow thus determined was over lumbar 6 and 7, and sometimes sacral 1. Results indicated, therefore, that most if not all myelinated fibers in the articular nerve were afferent. The temporal dispersion allowed the recording of numerous elevations corresponding to fibers conducting at rates of 8 to 85 meters a second. This fits with the grouping of fiber sizes determined previously. (3) The articular branch was severed from the sciatic. Spontaneous potentials were usually present. Trains of spikes were recorded following pressure on the capsule. The receptors stimulated appeared to be slowly adapting and were probably the Ruffini-type endings described previously.

45. Studies on germinal epithelium in ovarian transplants in mice. W. U. GARDNER and M. H. LI, Department of Anatomy, Yale University.

Previous studies from this laboratory revealed that ovarian transplants into the spleens of castrated male and female mice gave rise to granulosa cell tumors, luteomas, or mixed cell tumors (Li and Gardner, Science, 105: 13–15, 1947). Ovarian transplants into the subcutaneous tissues or testes rarely or never became tumorous in either intact or castrated hosts.

Non-tumorous proliferation occurred in ovarian grafts at all sites (testis, subcutaneous tissues, spleen and kidney), and differed depending upon the sites and endocrine status of the hosts. All well developed grafts were partially surrounded by a bursa-like space or spaces of variable sizes lined by epithelium. Transplants in all sites, except subcutaneously in intact hosts, showed tubular ingrowths of germinal epithelium into the graft. Cysts formed by germinal epithelium sometimes contained blood and the lining cells contained pigment (hemosiderin) indicating phagocytosis. Stages indicating transformation of germinal epithelial cells into macrophages were noted. Ingrowths of germinal epithelial cells appeared to transform into interstitial and granulosa cells. Subcutaneous grafts of ovary in intact males showed no germinal epithelial ingrowths and had "hypophysectomy-like" interstitial cells. Luteinized follicles were found only in grafts in females. Qualitative and quantitative differences in the gonadotrophic hormones are considered responsible for part of the activity of the germinal epithelium and cellular constitution of the ovarian grafts.

130. *Histological observations on the vitamin E-deficient rabbit heart.* Arthur J. GATZ, Department of Anatomy, Loyola University Medical School, and O. Boyd HOUCHIN, Department of Biochemistry, University of Louisville School of Medicine.

Previous studies by the authors have shown that degeneration areas occur in the cardiac muscle of rabbits reared on vitamin E-deficient diets. The present series of E-deficiency experiments in which vitamin D was supplied as cod liver oil in some series, and as Viosterol in other series, confirms our previous results. The electrocardiograms of the experimental animals consistently show a reversal of the QRS interval in lead I.

The portions of the heart which show necrosis of cardiac muscle are listed according to the frequency and severity of the lesion: papillary muscles, columnae carneae, apex of heart, ventricular walls and septum and infrequently atria. The Purkinje fibers appear to be unaffected.

The affected cardiac muscle fibers may show wide contraction bands, hyaline degeneration, basophilia with the appearance of numerous droplets in the muscle fiber (which fail to respond to acid fast dyes and are unstained by osmic acid) and finally complete necrosis caused by the coalescence of the aforementioned droplets.

90. *Sensory and parasympathetic nerve terminations in the nasal mucosa of the cat.* Edwin F. GILCHRIST and Kermit CHRISTENSEN, Department of Anatomy, St. Louis University School of Medicine.

The mucous membrane was taken from various parts of the nose of normal animals and of 2 groups of operated animals: one, in which each animal had a unilateral cervical sympathectomy; the other, in which each had the nerve of the pterygoid canal and the sphenopalatine nerves removed on one side. Sections were prepared by the activated protargol technic.

In the epithelium of normal and sympathectomized animals single nerve fibers, or sometimes plexuses of nerve fibers, with bulblike enlargements were observed coursing from the base of the epithelium toward the surface. Similar nerve terminations were also seen in the tunica propria. These nerve terminations are considered to be sensory and the nerve fibers reach the nasal mucosa as components of the nasal and ethmoidal nerves. A part of these nerve terminations disappear following removal of the nerve of the pterygoid canal and the sphenopalatine nerves.

In both normal and operated animals smaller nerve fibers with beadlike endings terminated in both glands and arterioles. Plexuses of these small fibers were observed paralleling and frequently entering the walls of the blood vessels. The terminations of the small nerve fibers are regarded as parasympathetic, especially because they are present in the sympathectomized animals.

53. *Differentiation on the basis of plasma cell formation of allergic from non-allergic inflammation in the cerebral cortex of the rabbit.* Robert A. GOOD, Department of Anatomy, University of Minnesota Medical School. (Introduced by Berry Campbell).

In an attempt to elucidate the nature of inflammatory cells and the cytomorphic mechanisms in the brains of hypersentitive and normal rabbits the following experi-

ments were carried out. Fifteen rabbits sensitized to egg white and 10 normal rabbits were used. The Romanowsky-stained air dried imprint method previously used was applied to this study. In both sensitive and nonsensitive rabbits sections of micro-lesions and imprints of the inflamed cerebral cortex were studied at interval's of 6, 12, 18, 24, 36, 48, 72, and 96 hours after the introduction of egg white.

In these studies previous author's findings that the acute inflammatory cycle in the central nervous system of non-sensitive rabbits is similar to that seen in loose connective tissue and that hemic lymphocytes are a source of phagocytic cells in central nervous tissues were substantiated. In rabbits sensitized to the irritant the time scale of the inflammatory cycle was altered, the inflammation was more extensive, and production of marked plasmacytosis in the lesions was noted. Alterations of the glial cells were seen in both the sensitive and non-sensitive rabbits.

98. Vessels and nerves in the hypophysial stalk and median eminence in man. J. D. GREEN, Department of Anatomy, Wayne University. (Introduced by Gordon H. Scott.)

Three groups of vessels may be distinguished in the median eminence and hypophysial stalk: (1) arterioles and small arteries in the pars tuberalis possessing smooth muscle and elastic tissue in their walls; (2) tufted glomerular-like loops of capillaries, chiefly within the median eminence which project into connective tissue sheaths composed of reticulum fibres, collagen, and undifferentiated mesenchymal cells; (3) portal vessels similarly ensheathed passing distally to the adenohypophysis and containing some smooth muscle in their walls. Nerve fibres are associated with all 3 types of vessel. Fibres may be seen to accompany the vessels in the tuberalis; these lie close to the wall and appear to be mainly vasomotor. The tufted capillary loops show many irregular fibres in their sheaths which are believed to derive mainly from the hypothalamico-hypophysial tract. They terminate in delicate branches and in small knob and ring endings. Similar endings and fibres may be seen about the portal vessels. Some of these endings are probably vasomotor since they are seen to end on smooth muscle cells. Others terminate blindly within the sheaths and may be secretomotor or even secretory. Nerve endings of similar nature have been found in a wide variety of forms, associated with corresponding blood vessels. So far the sheaths have only been observed in man and the macaque.

107. An x-ray study of the growth and development of the sacrum of girls during puberty and early adolescence. William Walter GREULICH and Herbert THOMS, Department of Anatomy and the Brush Foundation, Stanford University and Department of Obstetrics and Gynecology, Yale University.

Lateral roentgenograms of the pelvis were repeated at intervals of approximately 12 months, over a period of several years, on 115 young White girls. In the majority of cases, the first films were made either shortly before, or soon after, the menarche and the observations were continued for from 2 to 5 years.

A comparison of successive films of individual girls shows that the increase in the length and the change in the shape of the sacrum proceed more slowly than the changes in the dimensions and configuration of the superior aperture of the same

pelvis. The sacrum seems to attain its adult shape and size within about 2 years after the menarche.

The paper is illustrated by lantern slides in which the sacral changes are compared with those which occur concomitantly in the size and shape of the superior pelvic aperture.

113. The localization of thyroxine containing radioactive iodine in the tissues of the rat. J. GROSS, Department of Anatomy, McGill University. (Introduced by C. P. Leblond.)

Radioactive iodine (I^{131}) was introduced into the thyroxine molecule by both chemical and biological methods. The chemical method yielded relatively large amounts of thyroxine (1–7 mgm), while with the biological method, microgram amounts of the labelled compound were obtained, these being within the physiological range.

Female albino rats were injected intravenously with the large dose of radio-thyroxine and sacrificed at 2 and 24 hours. The radioactive hormone was traced by Geiger counter and autographic methods.

A predominant role of the gastro-intestinal system in thyroxine metabolism was shown by these experiments. At 2 hours after the injection, nearly half of the administered dose had found its way into the lumen of the gastro-intestinal tract and at 24 hours the major portion of the injected dose was found in the feces. Butyl alcohol extractions showed that a considerable proportion of the radioactivity was present as thyroxine with the exception of the stomach contents in which the radioactivity was predominantly as the non-thyroxine form.

The relative concentrations of radio-thyroxine in over 30 organs and tissues following large and small doses will be reported.

64. Deviation of axial organs as a cause of sirenoid malformations. Peter GRUENWALD, Department of Pathology, Long Island College of Medicine.

A series of sporadic malformations in chick embryos of the first week of incubation shows ventral deviation of the notochord, or of all axial organs into the yolk sac or its derivatives. The deviated parts may disappear later on by degeneration of their tissues. This results in a defect of median organs which may be classified as a sirenoid malformation, usually of a low degree. In some cases there is fusion of normally bilateral parts in the midline. One of these cases resembles a human siren, 19 mm long, which was previously described.

This mode of origin of sirenoid malformations, as well as the degeneration of previously normal appearing tissues in the development of hereditary malformations of similar types, demonstrates that the embryogenesis of sirenoid malformations cannot be determined by examination of the fully developed condition.

123. Effect of aging on 17-ketosteroid output of normal males. Howard B. HAMILTON, Department of Anatomy, Long Island College of Medicine. (Introduced by George H. Paff.)

Urinary 17-ketosteroid values in 48–144 hour collections from normal males aging 20–73 years were studied. The procedure followed is Dobriner's method of acid

hydrolysis and continuous ether extraction. The extract is partitioned into acidic, phenolic (estragenic), and neutral (androgenic) fractions. After further purification, the 17-ketosteroids in the neutral fraction are measured colorimetrically.

The steroid values for 24 hours show a gradual but definite decline with aging. The average is 10.2 mgm (range 16.5–5.6; mode 9.1) for 11 men in the third decade; 10.7 mgm (range 22.8–5.2; mode 9.6) for 9 men in the fourth decade; 8 mgm (range 15.2–5.1; mode 7) for 11 men in the fifth decade; 5.9 mgm (range 10.4–3.5; mode 4.7) for 10 men in the sixth decade; 4.9 mgm (range 6.7–3.2; mode 4.1) for 7 men in the seventh decade; and 3.4 mgm (range 4.2–2.8; mode 3.3) for 3 men in the eighth decade.

The slow decline in average hormone output indicates gradual lessening of secretion of 17-ketosteroid precursors but at no age is there a sudden drop in titer. These data are inconsistent with postulates of an abrupt "male climacteric," but definite conclusions are not to be drawn until a complete study of these men with regard to testicular biopsies, urinary gonadotrophins, androgens and estrogens is made.

124. Growth changes induced by androgens in the connective tissues, sebaceous glands, hairs, muscle, and melanoblasts of the skin. James B. HAMILTON, Department of Anatomy, Long Island College of Medicine.

Kupperman (1944) reported that androgenic stimulation results in pigmentation of 2 cutaneous spots in hamsters. Histological study with Dr. William Montagna, using Mallory stains of the tissues of which this spot is composed, shows the following rapid changes and acceleration of numerous growth processes after androgenic treatment of ovariectomized hamsters: (1) Pronounced increase in vascularization. (2) Proliferation of hairs and increase in their size and pigmentation. (3) Greatly-augmented melanogenesis particularly in hair roots and regions surrounding hair follicles and sebaceous glands. (4) Many-fold increase in volume of sebaceous glands and marked fatty changes in their cells. (5) Enlargement of muscle fibers and fibrils of the panniculus carnosus accompanied by denser staining and accentuation of cross-striation. (6) Great increase in coarseness of fibers and fibrils in dermal collagenous tissues. The collagenous fibers appear light blue or gray, in contrast to their deep blue color in control animals, and after 1 week of treatment acquire orange-colored streaks that do not correspond necessarily with complete collagenous fibers. Cells in the dermis (particularly mast cells?) appear larger and in some areas are much more numerous. In some areas intercellular spaces are enlarged, suggesting that the amount of ground substance may be increased.

These changes induced by testosterone propionate are in the direction of the status of normal adult males, but with continued treatment the growth of some constituents surpasses that in normal males.

70. Embryology of the molossid bat, Eumops. George W. D. HAMLETT, Department of Anatomy, Louisiana State University School of Medicine.

Study of a series of embryos of the molossid bat Eumops has shown several points in which this relatively unknown neotropical family differs embryologically

from other bats. The uterus is bicornuate, but only the right horn and ovary are functional. The single blastocyst attaches in the extreme upper end of the right cornu and expands into contact with the endometrium on all sides. The first chorionic villi are in the region of the yolk sac. This early yolk sac placenta is soon replaced by an allantoic placenta, and the yolk sac degenerates; the definitive endotheliochorial placenta covers the entire surface of the chorion until a late stage in development and never becomes definitely discoidal. The inner cell mass reaches the surface of the blastocyst to form an exposed embryonic shield which is then enclosed by the overgrowth of amniotic folds.

In its exposed embryonic shield Eumops differs both from European bats, in which the trophoblast is described as roofing over shield and primitive amniotic cavity at all times, and from the phyllostomids and Megachiroptera in which the amniotic cavity forms by hollowing out of the entypic inner cell mass. The diffuse, endotheliochorial placenta apparently differs from all other bats, although one worker has claimed a discoidal, endotheliochorial placenta for 2 European vespertilionids. Even in the phyllostomids and Megachiroptera, which attach over the entire chorionic surface, the villi soon disappear except in the definitive placental disk. The small allantoic sac resembles that of the vespertilionids and differs from both the Megachiroptera, where it is large, and the phyllostomids, where there is only an urachus.

55. *Hematopoiesis in the bone marrow of rats recovering from nutritional anemia.* Christopher J. HAMRE, Department of Anatomy, University of Minnesota. (Introduced by Hal Downey.)

Sixty rats were made anemic by being fed a diet of cow's milk only. Seven of these were killed as untreated anemic control animals while 53 animals were fed 0.5 mgm iron and 0.003 mgm copper per gram body weight to stimulate recovery hematopoietic processes. The treated animals were killed at hourly intervals during the first 48 hours after feeding of copper and iron. Studies of blood values and of sections and imprints of the marrow of the femur and humerus of all animals were carried out.

The bone marrow of all untreated animals was hyperplastic and hematopoiesis of the homoplastic type was arrested through a failure of maturation of blood cells. The marrow responded to recovery treatment by the development of extravascular heteroplastic erythropoietic tissue, including numerous pronormoblasts. Development and proliferation of hemocytoblasts from reticular stroma cells was observed. The greatest development of the new erythropoietic tissue, the greatest number of instances of migration of normoblasts and granulocytes into the venous sinuses, and the highest values for total number of leucocytes, normoblasts, heterophiles and eosinophiles of the circulating blood were found for animals killed 18 to 26 hours after the first feeding of copper and iron. We regard this as the critical period of regenerative hematopoiesis. By 36 to 48 hours following beginning of therapy, heteroplastic erythropoiesis had disappeared and an active homoplastic erythropoiesis had been established.

99. *Quantitative analysis of the brain-isocortex relationship in Mammalia.* Pinckney Jones HARMAN, New York University College of Medicine and Georgetown University Brain Research Institute.

The volumes were estimated on stained and sectioned material by planimetric procedures. The values in cubic millimeters for total brain and isocortex respectively are as follows: Primates: Pan 81,400; 38,100. Papio 48,800; 22,400. Ateles 28,600; 13,800. Cercocebus 28,100; 15,300. Macaca 21,100; 10,600. Cercopithecus 19,500; 11,400. Cebus 17,200; 9,130. Perodicticus 2,930; 1,140. Lemur 1,980; 797. Galago 1,260; 577. Carnivora: Lynx 17,600; 7,310. Vulpes 11,500; 4,380. Cercoleptes 6,750; 2,650. Felis 6,690; 2,690. Canis (young) 5,630; 2,500. Mustela 4,470; 1,850. Putorius 776; 302. Ungulata: Tagassu 29,700; 8,390. Rodentia: Erethizon 4,320; 1,260. Cynomys 1,190; 341. Cavia 867; 243. Marsupialia: Didelphis 1,310; 162.

Curves have been constructed and regression formulae, t values and coefficients of correlation (r) have been computed for the log brain-log cortex relationship in Primates (including man), Carnivora, and Mammalia (using 1 representative from each Order). The r values are very high for Primates (0.9986) and Carnivora (0.9996), but only moderately so for Mammalia (0.7691). The indications are that the relationship within Orders is linear, although inspection of the primate plot reveals a tendency toward cortical maxima in the middle range, suggesting a curve of the second degree. Some other function (apparently para bolic) probably best obtains for the Class.

The above observations are consistent with the findings of previous investigators on the brain weight-body weight relationship in Mammalia.

131. *Histological changes in kidneys of choline-deficient rats.* W. Stanley HARTROFT, Banting and Best Department of Medical Research, University of Toronto. (Introduced by Charles C. Macklin.)

Young rats on a diet containing all known essential food factors other than choline, develop the lesion known as "haemorrhagic kidney." The present paper, confirming and extending reports of others[1, 2] concerning this renal acholinopathy, is based on histological observations of paraffin and frozen microsections of kidneys of 75 animals, for 7 days on such a diet, and initially weighing 35 to 45 gm. Animals were killed at daily intervals during this period. Survivors, then given a normal diet, were sacrificed on reaching maturity.

Previously reported lesions in the acute stage, including subcapsular haemorrhage, postglomerular capillary plexus congestion, proximal convoluted tubular necrosis and tubular cast formation at the cortico-medullary junction, were confirmed. Preceding the development of these lesions, intracytoplasmic, sudanophilic droplets in the proximal convoluted tubular epithelial cells were noted. The possible pathogenic rôle of this lipoid substance will be discussed. Alterations of the renal vascular flow, demonstrated by intra-arterial india-ink injections, are considered an important factor in the production of tubular necrosis.

Extensive cortical parenchymal destruction during the acute stage produces characteristic sequelae in the survivors attaining maturity. Lesions of nephrons resemble those of subacute and chronic glomerular nephritis in man.

[1] Christensen, K., Arch. Path. 1942, *34:* 633.

[2] György, P., and Goldblatt, H., J. Exp. Med., 1940, *72:* 1.

16. *The participation of various portions of the urinary bladder in normal and induced activity.* Erling S. HEGRE and E. H. INGERSOLL, Department of Anatomy, Medical College of Virginia, Richmond, Virginia.

A study has been made of the activity of the uriary bladder of the female cat under light nembutal anesthesia. Various points on the exposed surface of the bladder were marked by white spots. From a photographic record on 16-mm film, the point to point activity of the various regions was plotted. During each experiment, a continuous kymographic record of the total bladder response was also obtained.

A comparison was made of the activity observed in each quadrant of the lateral surface of the bladder during rhythmic contraction, after the intravenous injection of adrenalin, and on stimulation of the hypogastric nerves. Contraction and relaxation per unit length in the observed areas were compared as to amplitude, rate, duration and sequence.

All parts of the bladder may participate in a given spontaneous contraction but all regions may not begin to contract at the same time. On stimulation of the hypogastric nerves, all parts of the bladder under observation show an initial, unsustained contraction which is followed by a marked relaxation. After an injection of adrenalin, all parts of the bladder may not exhibit the same response. A sustained contraction has been observed in the proximal portion while all other parts of the bladder have relaxed.

56. *Effect of synthetic Lactobacillus casei factor on the blood changes induced by gastrectomy in the rat.* George M. HIGGINS and Olive R. JONESON, The Mayo Foundation; Dwight J. INGLE, Upjohn Laboratories.

Synthetic L. casei factor has proved effective in controlling certain clinical as well as experimental anemias. Granulocytopenia, induced by giving sulfonamides, was corrected by L. casei factor. Granulocytopenia, occurring spontaneously, was corrected by the factor. Neutropenias induced by bone marrow depressants, however, did not respond. Anemias, induced by changing the bacterial flora, improved by giving L. casei factor. Total removal of the stomach from rats incites microcytoses, lowered hemoglobin levels and lowered granulocyte counts. This report covers the results of giving L. casei factor to gastrectomized rats in which a microcytic hypochromic anemia had persisted for 5 months.

Twenty-four healthy adult male rats, weighing about 300 gm were selected. Sixteen were gastrectomized; 8 were unoperated. All were fed a purified diet, of sucrose, vitamin-free casein, corn oil and salt mixture. Five months after operation L. casei factor was provided in the diet, at a level of 100 μg per gm, for 14 days. L. casei factor was then given intraperitoneally (400 μg per day) for 14 days. Administration of L. casei factor, either in diet or parenterally did not change red cell count, red cell size or hemoglobin level of gastrectomized rats. Total number of granulocytes increased from 1,900 per mm^3 to 7,200 when the vitamin was given orally; and to 11,100 when given parenterally.

74. The mouse ovary and its adrenocortical functions. R. T. HILL, Department of Anatomy, Indiana University.

Large numbers of inbred mice (CHI strain) have served in these experiments. Ovaries were grafted in either the ears of donor animals (homografts) or in the ears or testes of male animals (heterografts). Adrenals were removed in 2 stages with approximately a 7-day interval between operations. Grafted ovaries were removed in some of the animals at various intervals following adrenalectomy. Death follows graft removal at intervals which are more variable and more extended than the survival interval in non-grafted adrenalectomized controls. When animals bearing grafts are adrenalectomized, part of the animals die within the period of survival of the controls. Others live more or less indefinitely, and at this writing, some graft bearing adrenalectomized animals are alive and in apparent good health at more than 250 days following adrenalectomy. The longest survival of adrenalectomized control animals (males and females) to date has been 29 days. Sodium chloride added to drinking water in concentrations of 1% to 2.5% does not prolong survival time of the controls.

24. The movement of human fetal brain cells in tissue cultures. M. J. HOGUE, Department of Anatomy, University of Pennsylvania.

Tissue cultures were made from the cerebellum of human fetuses whose crown-rump measurements varied from 27 mm to 185 mm. They were grown in roller tube and hanging drop cultures. The first cells to move out from the explanted tissue were small oligodendroglia cells which did not live long when alone. Later when granule cells and other nerve cells appeared the oligodendroglia cells lived and flourished in indentations of these cells and on their processes. This suggests a symbiotic relationship. Macrophages appeared at this time. These were soon followed by large nerve cells. In 10- to 12-day-old cultures Purkinje cells appeared and worked themselves free from the mass of migrating cells. They moved by means of their 2 large branched anterior dendrites. The long slender axon trailed behind. Other large cells, probably from the dentate nucleus, moved out by means of their processes which were numerous and much branched.

80. The inhibition of mitotic activity in the anterior hypophysis during pregnancy and after injections of gonadotropins. Thomas E. HUNT, Department of Anatomy, Medical College of Alabama.

Mitotic activity in the anterior hypophysis of 3-month-old ovariectomized rats can be increased from an average of 1.2 to 22.1 mitoses per mm^2 of section with 2 injections of 25 μg of estradiol benzoate (Progynon-B) made 48 and 72 hours before death. In pregnant animals of the same age such injections result in approximately the same mitotic activity (17.33, S.E., $\pm$ 3.27) if made on the fourth to sixth day of pregnancy, but if made on the tenth to thirteenth day, after the placenta is well established, mitotic activity is reduced to 4.35 $\pm$ 1.41. To test whether this inhibition of mitotic activity is due to gonadotropins, extract of pregnancy urine (Pranturon) and extract of pregnant mare serum (Anteron) were injected into ovariectomized animals which also received estrogen. Injections of 25 and 50 I.U. of the gonadotropins did not significantly inhibit the mitotic

activity caused by the estrogen, but with 4 injections of 150 I.U. of Pranturon the activity was reduced to 4.9 ± 2.2 and with 3 injections of 500 I.U. of Anteron, it was reduced to 3.8 ± .87. In comparison with the animals receiving estrogen only (average 22.1 ± 5.5), the probability of the difference being significant is less than .01.

92. *Hypothalamic obesity in the cat.* W. R. INGRAM, Department of Anatomy, State University of Iowa.

Although hypothalamic obesity has been experimentally produced and extensively studied in the rat, the occurrence of this type of obesity in cats has not yet been reported. After production of bilateral hypothalamic lesions of rather specific location cats display altered behavior which ranges from defensive attitude to actual savageness, and includes considerably increased avidity of appetite. Most of these cats will gain weight quite rapidly. This occurs to some extent even while they are fed the same measured diet on which their preoperative weights were kept constant. This period of gain may level off after some weeks during which the weight may have increased 400 to 1000 gm, or in extreme cases 2000 gm. If the ration is than increased 50% a long period of increasing obesity ensues. In the most striking case the weight increased from 3100 gm initial to 7800 gm. This obesity may be reduced by drastic restrictions of diet. The fat deposits, while general, are greatest in the omenutum and retroperitoneal regions. The hypothalamic lesions are quite restricted and confined to the region of the ventromedial nuclei. In most cases the latter are completely destroyed; the ventral portion may be preserved, however, in fat and savage animals. These cats appear to have a tendency to fatty alteration of the liver.

26. *Tissue cultures of cells from ascitic fluid.* James B. IVERS and C. M. POMERAT, Department of Anatomy, University of Texas, Medical Branch.

Cells from ascitic fluid were collected from the bottom of 250 ml bottles after centrifugation for 15 minutes at 1500 r.p.m. Hanging drop and roller tube cultures were prepared, using rooster plasma and extract from chick embryos incubated for 7 days. The supernatant for roller tube cultures consisted of 10 parts of ascitic fluid, 10 parts of Tyrode's solution and 1 part of embryonic extract. Observations were made both on living cells and of preparations stained with hematoxylin-eosin-azure.

Upon comparison, cultures from a patient with a diagnosis of scirrhous carcinoma of the stomach were found to resemble those obtained from a patient with cirrhosis of the liver in that both started with a collection of polymorphonuclear leucocytes, lymphocytes, monocytes, polyblasts and mesothelial cells. After 10 days both resulted in pure cultures of spindle cells resembling fibroblasts.

In contrast, a patient with a diagnosis of intra- and extra-cystic papillary cystadenoma of the ovary and another with an adenocarcinoma of the ovary with metastasis to the peritoneum both showed organized epithelial tissue bits initially and these developed into well-formed cysts, suggesting a definite tendency to gland formation. In these 2 cases no spindle cells were seen.

Work in progress is attempting to define the effect of gonadotropins, sex hormones, anti-organ immune sera and x-rays on such cellular aggregates *in vitro*.

126. Intrasplenic injections of alloxan in the rat.[1] Ralph G. JANES, Department of Anatomy, State University of Iowa.

Alloxan is apparently effective in damaging the pancreatic islands for a few minutes only following its injection. In an attempt to determine whether the liver was responsible for the conjugation or destruction of alloxan, this substance was injected intrasplenically. Of 16 non-fasted rats which lived following intrasplenic injections of alloxan (50–150 mg/kg), 5 became severely diabetic. Of 10 rats which received similar amounts of alloxan intraperitoneally, 2 became severely diabetic. Alloxan was most effective in producing a severe diabetes when the rats were fasted 3 days before they were injected. Unless alloxan was injected very slowly (1/100 cm^3/min.) the rats failed to survive intrasplenic administration of the drug. When injected rapidly, infarcts in the liver were common or the liver cells showed a marked vacuolation, caused in part by a hydropic condition. The data indicate that alloxan was just as effective in producing diabetes when given intrasplenically as when given intraperitoneally. However, it is difficult to compare the effectiveness of intrasplenic and intraperitoneal injections, since it has been shown that the intravascular administration of alloxan is more effective than other methods and because intrasplenic injections might be considered comparable to intravascular injections. Nevertheless, it may be concluded that alloxan is not inactivated to any great extent by the liver.

[1] This work was supported in part by the Hoffmann-LaRoche Co.

94. Effect of intermittent exposure to 30,000 feet simulated altitude on brain structure. Arthur V. JENSEN and William F. WINDLE, Departments of Anatomy, University of North Carolina and University of Washington.

Young adult male guinea pigs were subjected to conditions simulating an altitude of 30,000 feet in a decompression chamber 6 hours daily and 6 days weekly for 100, 150, 200 or 250 hours. At appropriate times, the experimental animals and controls were killed by perfusion through the vascular system with a formaldehyde fixing solution to preserve the nervous system and other organs in situ. Histological technique was carefully controlled.

The animals responded to reduced barometric pressure by maintaining a quiescent state, usually with episodes of physical distress, collapse and, apparently, unconsciousness. After removal from the chamber, comparison with controls rarely revealed significant behavioral or physical differences. No permanent neurological defects could be observed. Some weight loss occurred.

Focal areas of degeneration occurred in the vermis of the cerebellum of all animals of the 200- and 250-hour groups and in 3 of the 7 animals studied in the 100- and 150-hour groups. Elsewhere in the brain, small areas of cell shrinkage or (less commonly) impaired staining occurred, fortuitously, it seemed. For the most part, brain tissues appeared to be normal and most regions looked exactly like comparable regions in the controls. The focal areas of degeneration or other structural change may have resulted from vascular stasis; marked leucostasis was encountered in the 250-hour group.

50. A study of mitochondria in dry smears of embryonic blood.[1] Oliver P. JONES, Department of Anatomy, University of Buffalo.

Studies on the megaloblast-normoblast problem have been made for a number of years (Jones, '34, '35, '38). During the course of these investigations it was necessary to study material similar to that which Doan et al. used when formulating their theory for erythropoiesis. Dry smears of blood from chick blastoderms revealed that both the cytoplasm and nuclei were not as devoid of structure as described by Doan et al. A comparative study of dry smears and supravital preparations showed a striking correlation between the pale or yellowish areas of hyaloplasm in the basophilic cytoplasm and the distribution of mitochondria. Because of this it was tentatively concluded that the so-called hyaloplasm represented negative images of mitochondria. More extensive studies were conducted on primitive erythroblasts from 11-day rat embryos by means of various cytological, cytochemical and optical techniques. It was found that freshly dried smears of embryonic blood cells preserve cytoplasmic detail to an extraordinary degree. Light areas in basophilic cytoplasm previously described as hyaloplasm or paraplasm represent, for the most part, the negative images of underlying mitochondria. Cytochemical techniques reveal that the light areas (mitochondria) are relatively rich in lipoid material and contain a carotenoid pigment — probably vitamin A. Cytoplasmic basophilia is due to ribonucleoprotein. Phase microscopy makes it possible to study structures in unstained blood cells which are usually hidden in routine preparations.

[1] Aided by The Dr. Grant T. Fisher Fund.

68. Observations on the differentiation and connections of specialized conducting tissue in the human heart. Cornelius T. KAYLOR and Jane S. ROBB, Departments of Anatomy and Pharmacology, Syracuse University College of Medicine.

Serial sections of 4 human fetal hearts aged 15½ weeks, 4½, 5½ and 8 months, were studied histologically to determine the differentiation and connections of A-V nodal and bundle tissue.

Nodal and bundle cells could be recognized in the youngest heart mainly by differential staining reactions. The topography of the definitive node and bundle is discernible at this early time. In the older hearts, conducting tissue showed distinct cytological and staining characteristics. The location of the A-V node and the bundle is similar to that described for rodents, carnivors, ungulates, and primates including the adult human. In the auricular septum, nodal cells merge imperceptibly with auricular muscle cells. In the ventricular septum, bundle cells merge with myocardial cells. Numerous septal connections, not in accord with usual descriptions, were observed in all hearts. The absolute size of the A-V node and bundle seemed to be maintained throughout development. No myocardial tissues were observed to be contributing to the specialized conducting tissues.

Sections of 2 4½-month fetal hearts were studied for nerve supply to A-V node. Preliminary examinations show nerve fibers to cardiac vessels and structures above the coronary sulcus, few if any nerve endings in conducting tissue.

37. *Another approach to the problem of efferent fibers in dorsal roots.* Donald L. KIMMEL and Elizabeth K. MOYER, Department of Anatomy, Temple University School of Medicine.

In 12 Wistar rats, the central segments of sectioned dorsal roots were anastomosed end-to-end with arterial sleeves to prevent the ingrowth of regenerating fibers. At 2 to 18 weeks after the operation, the cord with the anastomosed roots as well as the peripheral stumps of the sectioned dorsal roots with their ganglia were removed under ether anesthesia. Small sections were taken from the anastomosed roots for osmic fixation. The remaining tissue was fixed in formol-acetic-alcohol and impregnated by Bodian's protargol method.

In studying the serial sections, the anastomosed roots of 6 animals were found to be contaminated by aberrantly located ganglion cells, intradural anastomoses, or regenerating fibers from the surrounding roots. In all the uncontaminated anastomosed roots of the remaining animals a few, fine, normal-appearing fibers were found. The intact fibers were sparse and scattered throughout the roots. At the cord-rootlet junction they lay in a position corresponding to the small fibers of a normal rootlet.

11. *Anatomical relations of possible significance in the production of the scalenus anticus syndrome.* Homer D. KIRGIS, Department of Anatomy, Tulane University, School of Medicine, and Division of Neurosurgery, Ochsner Clinic, and Adrian F. REED, Department of Anatomy, Tulane University, School of Medicine.

One hundred and twelve dissections have been made to determine anatomical relations of possible significance in production of the scalenus anticus syndrome. The etiological significance of a cervical rib or an elongated cervical transverse process is well-known but the majority of cases do not present these anatomical variants. Additional anatomical features which seem important include the relations of the scalenus anticus muscle at its origin, the presence of a scalenus minimus muscle or a vertebro-costal ligament, the relations of the scalenus medius muscle, and the sites of insertions of the scalene muscles. Each subject revealed muscle fibers joining the upper portion of the scalenus anticus from an origin along the inferior and posterior aspects of the intervertebral canal. These, upon contracting, may exert pressure on the nerve located immediately inferior to their points of origin. Gross and microscopic examination demonstrated many muscle fibers to have their origins from the sheaths of adjacent nerves. The scalenus minimus muscle was present in 55.4% of the cases and the vertebro-costal ligament in 25.9%. The widths of the insertions of the scalenus anticus, minimus (or vertebro-costal ligament) and medius muscles and the intervals between the insertions of these structures were measured. Although certain anatomical relations may favor the development of the scalenus anticus syndrome, it is apparent that no variation from the normal anatomy need be present for its occurrence.

49. Intraepithelial granular cells in urinary tracts of rats infested with Trichosomoides crassicauda. Hadley KIRKMAN, Department of Anatomy, Stanford University.

The cells are absent in the transitional epithelium of uninfested rats. Their origin and homologies remain uncertain. On the basis of staining reactions and other tests applied to fresh and fixed material it is believed that they are not mast cells, Russell body cells, serous cells, mucous cells, plasma cells, fibrocytes, macrophages or hemophages.

They exhibit certain similarities to eosinophilic myelocytes, globular leucocytes (Schollenleukocyten), and "secretory leucocytes" of fishes. Unlike the myelocyte (but like the mast cell) they exhibit no mitochondria with Janus green, anilin acid fuchsin or iron hematoxylin. Their granules stain well, but not metachromatically, with alkaline toluidine blue and thionine. With eosin they stain but lightly. Pale lavender with Wright's stain they color brilliantly with azocarmine. They are negative to indulin and to Nadi reagent. On the other hand, like eosinophilic myelocytes their granules assume a pale yellow coloration with neutral red, are highly refractile and give a variable peroxidase reaction.

Unlike certain descriptions of "globular" and "secretory" leucocytes they contain no iron or hemoglobin and exhibit no siderophilia. Their granules are less labile and less eosinophilic.

In view of lack of precise data concerning the nature and significance of "globular leucocytes" it is concluded tentatively that the 2 cells may be related and that the possibility of a causal relationship between roundworm infestation and Schollenleukocyten might well be investigated.

51. In vivo acceleration of fibroblastic mitosis by injection of extract of inflamed tissue. Fred KOLOUCH, Department of Surgery, University of Minnesota Medical School.

The usual fibroblastic mitotic activity commencing 24 hours following induction of sterile inflammation by the subcutaneous injection of egg white in the rabbit led the author to investigate the effect of the subcutaneous introduction of a saline extract of connective tissue inflamed for 24 hours. The extract was made from connective tissue removed from a rabbit 24 hours following subcutaneous injection of egg white. 0.1 cm^3 of the extract was injected subcutaneously at multiple points on the back of normal rabbits. Biopsies prepared as dry tissue spreads (stained with Wright-Giemsa) were studied at 2, 4, 8, 12, 16, 24, 48, 72, and 96 hours following injection. An acceleration of the entire inflammatory cycle occurred as compared with the response to egg white or an extract of normal rabbit connective tissue. An increased number of polymorphonuclear leukocytes as well as an earlier appearance of hemic lymphocytes followed by a rapid transformation of lymphocytes to macrophages occurred. The fibroblasts showed an earlier nuclear hyperchromasia and cytoplasmic basophily. By 24 hours mitotic activity of the fibroblasts was considerably increased over that of the controls.

86. Gigantism produced in normal female rats by chronic treatment with pure pituitary growth hormone. III. Pituitary-cytological changes. Alexei A. KONEFF and Choh Hao LI, Division of Anatomy and Institute of Experimental Biology, University of California.

Normal plateaued female rats were injected for 437 days with pure growth hormone. Non-injected rats of the same age were used as controls. The average weight of the pituitaries was increased, but the increase was not proportional to the body weight gain. Pituitaries were examined in 4 micra celloidin sections stained with Mallory-Azan. Acidophils were markedly decreased in number. Planimetric measurements showed a significant decrease in size of acidophils (mean of injected group 156 ± 6, control group 189 ± 7). There was considerable depletion of granules in these cells. The extent of the changes in the acidophils paralleled the body weight increase, being most pronounced in the animals showing the greatest gain. The findings must be interpreted as indicating decreased functional activity of acidophils — the cells believed to be the site of growth hormone production. The basophils of six of eight pituitaries did not manifest any significant deviation from the normal picture; castration-like changes were present in the basophils of the remaining two pituitaries. No changes were noted in the intermediate or posterior lobes of the pituitaries.

100. New apparatus for the study of cardiac and circulatory ballistics. Vernon E. KRAHL, Department of Anatomy, Wayne University. (Introduced by F. Gayor Evans.)

Two vertical ballistocardiographs are described. One is a simple appartus consisting of a bath scale, writing lever and kymograph. It does not give quantitative information concerning heart output, but produces the characteristic ballistic waves and records changes in the size of impacts. It has proved satisfactory in the teaching laboratory as a means of demonstrating the effects of exercise and certain drugs upon cardiac output. In the second and more sensitive instrument, the recoils of the body produce deformations in resistance strain gages which are arranged as a Wheatstone bridge. The unbalance of the bridge is amplified and the signal fed into a cathode ray or other oscillograph. Records obtained with each ballistocardiograph are illustrated and discussed.

31. General conclusions from an experimental study of the cerebral cortex of the albino rat. Wendell J. S. KRIEG, Institute of Neurology, Northwestern University Medical School.

One hundred minute lesions were made in the cerebral cortex and thalamus of the albino rat, the brains prepared by the Marchi method, and the revealed connections stereographically reconstructed. Findings were then synthesized and described on the basis of anatomical cortical areas, permitting the following general conclusions:

Applications of the Marchi method after cortical lesions does not distinguish between thalamocortical and corticothalamic fibers. Every thalamic nucleus studied

sends fibers to the cortex and none studied sends fibers elsewhere. Internuclear connections are very scant.

Nearly every area sends nonthalammic projection fibers to the cerebral peduncle or further. These pass to nearly every main division of the brain, except striatum and hypothalamus. Most areas have homeotopic callosal connections systematically arranged but no heterotopic connections were observed.

Association fibers are few, and are mostly intracortical, but the concept of an undifferentiated feltwork is not sustained. Many associational connections are directed caudomedially so that the retrohippocampal region receives from a widespread expanse.

All categories of connection indicate a high degree of point for point specificity and the fibers are arranged discretely and orderly in the fiber accumulations. Exceptions exist. Destruction of the outer layers of the cortex or undermining does not produce substantial cell loss of the remaining layers, due perhaps to the numerous intact collaterals. Retrograde degeneration is not a fruitful method for cortical investigations.

96. *Neuroectodermal neoplasms of borderline dysplastic character. A survey of different types and their relationship to normal histogenesis.* Hartwig KUHLENBECK and Webb HAYMAKER, Army Institute of Pathology, Washington, D. C. and Department of Anatomy, Woman's Medical College of Pennsylvania.

In a survey of the tumor material available at the Army Institute of Pathology, the following 5 types of neuroectodermal neoplasms were found to be of special interest as examples of disturbed normal ontogenetic processes.

1. Atypical subependymal spongionueroblastoma associated with tuberous sclerosis (Bourneville's disease).

2. Neoplastic transformation of hyperplastic corpus pontobulbare and subependymal cell plate in the wall of the fourth ventricle (spongioblastoma subependymale diffusum).

3. Dysplastic, persistent transitory embryonic superficial granular layer of the cerebellum associated with cerebellar neuroblastoma or spongioneuroblastoma containing anaplastic Purkinje and other neuronal elements.

4. Transformation of dysplastic superficial granular layer of the cerebellum, associated with ectopic Purkinje cells, into glioneuroma with neuronal elements of Purkinje cell type.

5. Transformation of the margin of a dysplastic optic cup into a glioneuroma with neuronal elements of pyramidal cell type, filling part of the anterior half of the eye bulb.

The significance of the subependymal cell plate as a potential source of diverse typical neuroectodermal neoplasms has already been pointed out in a previous communication. In addition, the present series shows that this structure may be involved in dysplastic processes of borderline neoplastic character. Other embryonic structures which may undergo similar transformations are the corpus pontobulbare, the transitory superficial granular layer of the cerebellum, and the margin of the optic cup.

91. Reactions of interstitial and capsule cells in autonomic ganglia to various stimuli.[1] Albert KUNTZ and Normal M. SULKIN, Department of Anatomy, St. Louis University School of Medicine.

Hyperplasia of the interstitial tissue, including the capsule cells, in autonomic ganglia has been induced in experimental animals (cats and dogs) by prolonged faradic stimulation of preganglionic fibers and by periganglionic application of alcohol. It is also a common phenomenon associated with various pathologic states in man. The cells in question undergo amitotic division.

Under conditions of hyperplasia, some interstitial cells assume spheroid forms and appear to be devoid of processes. Within the ganglion cell capsules some cells become separated from the capsular wall. Some of these gradually become spheroid and encroach upon the ganglion cells. At the points of contact of the spheroid cells with a ganglion cell concavities arise in its peripheral cytoplasm. These concavities gradually become deeper and the spheroid cells sink into the ganglion cell body. This involves dissolution of some of the cytoplasm of the ganglion cell apparently due to a cytolytic process.

[1] This work has been supported by a grant from the Life Insurance Medical Research Fund.

33. Some morphological features of the human cerebellum. O. LARSELL and W. A. STOTLER, Department of Anatomy, University of Oregon Medical School.

Two adult human cerebella, 1 with unilateral agenesis of one cerebellar hemisphere and the other with greatly reduced anterior lobe, and several normal child cerebella, show clearly the spino-cerebellar and trigemino-cerebellar tracts in simple relations approaching those of lower vertebrates. The 2 tracts lie close together and form a commissure corresponding to the cerebellar commissure of lower forms. In the case on one-sided agenesis the tracts are shown to be chiefly crossed. The dorsal nucleus of Clarke, the dorsal spino-cerebellar tracts, lateral cuneate nucleus and direct cuneo-cerebellar tract appear only on the side homolateral to the intact hemisphere. A band of fibers apparently corresponding to the lateral commissure of lower vertebrates extends medially from the base of the floccular peduncle and decussates across the midplane. The arciform nucleus of the medulla oblongata is connected with the contralateral side of the cerebellum by fibers which course near the olivo-cerebellar tract. A small homolateral connection also is present. The stria medullares of the fourth ventricle floor connect the arciform nucleus with the contralateral corpus pontobulbare. No ventral external arcuate fibers, as usually described, are present. Olivo-cerebellar and ponto-cerebellar connections appear to be predominantly contralateral, while reticulo-cerebellar connections are homolateral.

109. The effects of partial starvation on somatotype. Gabriel W. LASKER, Department of Anatomy, Wayne University, and Laboratory of Physiological Hygiene, University of Minnesota. (Introduced by Ernest D. Gardner.)

Under the direction of Dr. Ansel Keys and his co-workers at the University of Minnesota, 34 volunteers were limited to a diet averaging 1,760 calories daily for 24 weeks. During this period they lost an average of 24% of their body weight.

Standardized nude photographs taken at the beginning and end of this period were made available to the author for study. Somatotyping techniques have been applied to these photographs to see whether so-called "constitutional" traits were influenced by changed dietary conditions.

Though partially starved individuals do not look identical to habitually lineal individuals, application of somatotype criteria proved practicable. Using 2 different codifications of Sheldon's technique of somatotyping, the majority of traits are shown to change in most of the individuals. Every individual shows a noticeable change in somatotype. Only in the wrists and ankles is there ordinarily little change. Similarly, preliminary studies by 5 somatotypers working under the direction of Dr. James Andrews in Professor Hooton's laboratory at Harvard confirm the findings of changed somatotype in every individual. Furthermore, 17 measurements taken by Sheldon as indicative of somatotype all show a tendency to decrease in the present series, most of them in every individual. The technique of somatotyping as applied in this study would thus seem to be a better measure of nutritional state than of inherent constitution.

128. Protection against alloxan diabetes. Arnold LAZAROW, Department of Anatomy, Western Reserve University.

Glutathione, cysteine, or thioglycolic acid previously have been reported as protecting rats against diabetes when these sulfhydryl compounds were administered intravenously prior to a diabetogenic dose of alloxan.

In the present study British Anti-Lewisite (BAL) was tested for protective action against alloxan diabetes. BAL is a disulfhydryl compound which can reactivate sulfhydryl groups of enzymes, previously inactivated by heavy metals. When BAL was injected in doses of 0.36 mM/kg immediately preceding a diabetogenic dose of alloxan, all rats were protected. However, when doses of 0.75 mM/kg of BAL were administered 5 minutes after alloxan, all rats developed diabetes. The diabetogenic action of alloxan may be due to its inactivation of essential sulfhydryl groups of enzymes within the beta cells. The failure of BAL to protect against diabetes when it was administered 5 minutes after alloxan may be due to an irreversible inactivation of sulfhydryl groups by alloxan.

Methionine, in doses of 2.9 mM/kg did not protect against alloxan. Thiourea, in doses of 7.5 mM/kg followed by alloxan, 40 mg/kg, proved acutely toxic. When nicotinamide (7.5 mM/kg) was injected intravenously immediately preceding alloxan (40 mg/kg), 5 of 18 rats developed diabetes. When alloxan alone was administered intravenously (40 mg/kg), 20 of 21 rats developed diabetes.

122. Factors influencing steroid hormone action in rats and mice. James H. LEATHEM, Department of Zoology, Rutgers University.

The route of administration is a determining factor in the response of 22-day-old rats to a single injection of 0.25 mg of testosterone propionate. After 72 hours the seminal vesicles are increased 3 fold when the intramuscular and subcutaneous routes are used but only slight stimulation follows intraperitoneal injection. In the mouse, a similar difference in injection routes is shown when the adrenal X zone is the criterion. Furthermore the volume of oil does not influence the response to a single injection.

Extending the injection period to 10 days it was found that the daily subcutaneous injection of 0.3 cm^3 sesame oil into 22-day-old mice would modestly suppress seminal vesicle weight. Therefore, the 0.25 mg total dose of testosterone proprionate was retested by dividing in into 10 daily subcutaneous injections given in 0.1 and 0.4 cm^3 of oil. The increase in seminal vesicle weight was roughly 3 fold in both groups, thus simulating the single injection in degree of response and in an absence of the oil effect. Unlike the single injection in which the intraperitoneal route was not effective, the intraperitoneal injection of 0.025 mg daily for 10 days caused a 250% increase in seminal vesicle weight and testis weight was only slightly influenced in comparison with the subcutaneous route. Therefore, the mode of administration and experimental period are effective agents in influencing results.

120. Detached respiratory squames from the lung of the albino mouse. Charles C. MACKLIN, The University of Western Ontario Faculty of Medicine, London, Canada. (See demonstration.)

If through a drop of physiological saline the fresh collapsed albino mouse lung be gashed repeatedly and the exuding mixture spread on a slide, dried and stained as for blood with Wright or Giemsa, there appear numerous thin cell derivatives which are identified as the desquamated respiratory squames or plates of Kölliker. The appearance is widely varied, and one cannot say that the average squame is either definitely nucleated or unnucleated; but rather that the nucleus has undergone profound change, tending to merge with the cytoplasm and to become extremely thin and pale-staining. Often its position is indicated, if at all, only by a less weakly stained central area. Frequently a sunburst form is presented, the nuclear substance seeming to be continued as radiations into the cytoplasm. Often an arc of the nuclear circumference forms part of the boundary of the squame. With ordinary section stains, the squames show little or no color. In fresh mounts, with weak illumination, they may be described as ghostlike. Trypan blue granules, in the chronically stained mouse, were seen in both fresh and fixed squames, so confirming the finding of John L. Bremer. Failure to see squames in ordinary sections is due to their extreme thinness and their frequently having been exfoliated, to appear only as debris. In the albino rat analogous, usually completely anuclear, squames were seen.

28. Sulfhydril groups, lithium, and the motility of human spermatozoa. John MACLEOD, Department of Anatomy, Cornell University Medical College, New York, N. Y.

Evidence is presented that the motility of human spermatozoa is dependent upon the integrity and lability of sulfhydril groups in certain enzyme systems. The addition of inhibitors such as Cu and arsenicals which are known to affect the integrity of sulfhydril groups, results in destruction of motility. Cysteine, glutathione and BAL protect the motile activity of the spermatozoa against these inhibitors and in certain circumstances, may reverse the loss of motility induced by the previous addition of the inhibitors.

The Li ion in very low concentration also has a profound effect in destroying the motility of human spermatozoa. The nature of this action is still under investigation. In all cases of loss of motility (Cu, arsenicals, or Li), a marked depression of glycolysis is also apparent.

14. The relation of separately innervated areas of muscle to amount of shortening and strength of contraction. J. E. MARKEE, W. W. THOMPSON, and S. O. THORNE, Jr. Department of Anatomy, Duke University School of Medicine.

We have previously reported that areas of a muscle innervated by separate branches of a nerve contract separately on electrical stimulation of individual branches, and have called attention to the pattern of branching of the nerves supplying long limb muscles of humans.

The present report deals with the long muscles of the thigh acting over both the knee and hip joints. In the human we have investigated the relation of muscle fascicles to each other and to the fibrous skeleton of the muscle. In the dog we recorded the contraction of separately innervated parts of these individual muscles.

It appears that these long thigh muscles are divided into 2 fundamental groups: those in which fibers are arranged in parallel e.g. rectus femoris, semimembranosus, in which stimulation of additional separate branches increases primarily the strength of contraction without affecting materially the amount of shortening; and those in which the fascicles are arranged essentially in series, and in which stimulation of additional separately innervated parts increases primarily the amount of shortening rather than the strength of contraction. The semitendinosus is a muscle of this type resembling structurally and functionally a two-bellied muscle, the two superficial bellies attaching to an intervening tendinosus inception. Functionally the sartorius behaves similarly; separately innervated areas produce additional shortening by contracting in series. However, in the sartorius there are no macroscopic septa dividing these areas.

106. On the embryology of levator ani muscle. Cecil P. MARTIN and Richard M. H. POWER, Department of Anatomy, McGill University.

A study of comparative anatomy, human embryology and some human malformations suggests that the pubo-rectalis should be interpreted as the caudal continuation of the ventral longitudinal muscle band which forms the depressor muscles of the hyoid bone, the sternalis and the rectus abdominis muscles. The ano-vaginal cleft is the lower end of the Linea Alba separated from the abdominal part by the development of the pubis. This interpretation contributes to an understanding of some anomalies.

102. Basic types of the arterial blood-supply of the supramesocolonic organs.[1] Nicholas A. MICHELS, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

Complexity of vascularization of liver and gallbladder is due primarily to site of origin, secondarily to mode of terminal (extra-hepatic) branching of the 3 major

arteries to the liver, viz. right, left and middle hepatic (latter for quadrate lobe). Patterns varied to such an extent that in the 200 bodies investigated no 2 are the same. Analysis shows that any sample no matter how complex falls into one of the following 10 basic types.

The celiacal hepatic supplies: (1) Right, left and middle (textbook type). (2) Right and middle hepatic — left replaced from left gastric. (3) Left and middle hepatic — right replaced from superior mesenteric. (4) Middle hepatic — right replaced from superior mesenteric, left from left gastric. (5) Right, left, middle — left hepatic small, hence an accessory left hepatic from left gastric. (6) Right, left, middle — right hepatic small, hence an accessory right hepatic from superior mesenteric. (7) Right, left, middle — all 3 being small there is an accessory right from superior mesenteric and an accessory left from left gastric. (8) Combination pattern of an accessory right hepatic with a replaced left hepatic or an accessory left hepatic with a replaced right hepatic. (9) Celiacal hepatic absent — entire hepatic trunk from superior mesenteric. (10) Celiacal hepatic absent — entire hepatic trunk from left gastric.

Basic arterial patterns will be presented of the supraduodenal, retroduodenal (posterior superior pancreaticoduodenal), dorsal pancreatic (superior pancreatic) and transverse pancreatic (inferior pancreatic).

[1] Aided by a grant from the American Philosophical Society.

18. Histochemistry of the sebaceous glands of the rat. William MONTAGNA, Department of Anatomy, Long Island College of Medicine, Brooklyn, New York. (Introduced by James B. Hamilton.)

Rectal and skin sebaceous glands were used in this investigation. The rectal glands are larger, more complex, and more active than those of the skin, but histochemically they are essentially alike. The studies discussed here apply to both types of glands.

Alkaline phosphatase is abundant in sebaceous glands, especially along their peripheries. Ribonucleic acid is present especially in the immature sebaceous cells.

Lipids are visualized by the application of several techniques. (a) Phospholipins along the periphery of the glands are shown by the Smith-Dietrich method and by the use of Sudan black (Baker, '44). (b) Nile blue sulphate reveals large amounts of unsaturated triglycerides in the larger acinar cells and sebum. (c) Birefringent spherocrystals are found in the sebum and cells undergoing sebaceous degeneration. These crystals are removed by solvents and do not form digitonides. Since the anisotropic material is weakly Schultz positive, perhaps it represents cholesteryl esters. (d) In addition to these lipids the glands contain a substance which is stained with Sudan black-B after prolonged immersion in solvents at 60°C. This substance may represent the saturated, monobasic, high molecular alcohol (eicosyl), suggested by Ameseder ('07) and Melczer and Deme ('42).

21. The distribution of alkaline phosphatase in the developing teeth of the 4-day rat. Anna MORSE and Roy O. GREEP, Harvard University School of Dental Medicine.

We find here favorable material for studying the precise localization and distribution, and the relative concentration of this enzyme in tissues specialized for the purpose of mineral deposition. The substrate used was sodium glycero-phosphate plus 0.0015 M magnesium. The pH was varied by half units from 7 to 11. Incubation time varied from 7½ minutes to 24 hours.

Alkaline phosphatase was demonstrated in all the dental tissues except the basal formative end of the enamel-organ and the cytoplasm of the pre-ameloblasts. However, its apparent distribution and concentration was greatly influenced by the pH of the substrate, period of incubation, and stage of development and differentiation of the tissue. Optimal circumstances for different tissues were not uniform. For instance, the stratum intermedium after an incubation period of only 1 hour was positive to a variable degree over a fairly wide range of pH and strongly positive over a much narrower range. The stellate reticulum near the outer enamel epithelium remained negative after much longer periods of incubation becoming positive only after 4 or more hours and within a narrow pH range. The cytoplasm of the secreting ameloblasts was never more than weakly positive under any circumstances.

From serial sections through longitudinal and cross sectional planes, the localization and concentration of the enzyme in areas of comparable differentiation was found uniform irrespective of mesial, distal, buccal, or lingual position.

25. Tissue cultures of adult human sympathetic ganglion cells. Margaret R. MURRAY and Arthur P. STOUT, Department of Surgical Pathology, Columbia University. (Introduced by Raymond C. Truex.)

Portions of 22 sympathetic ganglia obtained surgically from 19 adult human individuals were cultivated in lying-drop cultures for a period of several months. Though many cells remained anchored in the explant, a considerable number migrated away into the culture medium for a distance of a few millimeters. These migratory cells resembled fetal and regenerating ganglion-cells in many respects. They maintained a conspicuous polarity in the absence of normal functioning. Their content of Nissl substance, nuclear chromatin, pigment, neurofibrils and Golgi bodies was variable and often atypical. The emigrated ganglion-cells did not organize themselves into a tissue or form synaptic relationships, although they might establish chance associations with one another or with satellite cells or capillaries.

Ganglion cells remaining in the explant habitually produced branching nerve fibers which extended for an average of 2 mm into the medium, and survived for months. They were often closely invested by Schwann cells which migrated and divided in a manner similar to that which they adopt in the developing animal. When the newly grown nerve fibers were severed in vitro, many of them regenerated. The latent period before the beginning of neurite growth and ganglion cell migration varied significantly among ganglia from different individuals.

53. Histopathological effects of plutonium, fission products, fast and slow neutrons, and x-rays on the thymus.[1] Raymond G. MURRAY, Department of Anatomy, Tufts College Medical School. (Introduced by William Bloom.)

The qualitative effects of x-rays on the thymus were roughly comparable in several hundred mice, rats, rabbits, guinea pigs, and chickens, agreeing essentially with the reports in the literature. The extreme sensitivity of thymocytes to doses near the LD50/30 days paralleled the sensitivity of lymphocytes in the spleen; epithelium, prominently revealed in both cortex and medulla, was insensitive. Regeneration, nearly complete a month after LD50/30 days, was primarily from medullary reticular cells and surviving lymphocytes. Evidence that epithelium participated in lymphocyte formation was suggestive but inconclusive. Changes resembling Hassall's body formation occurred in cortical epithelial cells which possibly had been phagocytes. Mice had small but otherwise normal thymuses after 20 daily 80 r treatments (single LD50/30 days about 500 r). The effects of fast and slow neutrons resembled those of x-rays.

Beta emanation from strontium^{89} and yttrium^{91} (2–5 μc/g), concentrated in the sternum after parenteral administration, depleted adjacent thymus lobules. Inhaled cerium-praseodymium (300-day) and yttrium^{91} (200 μc/rat) caused more damage in adjacent thymus than in the heavily contaminated lung. Following zirconium^{93}–columbium^{93} and barium^{140}–lanthanum^{140} (3–5 μc/g), the rat thymus was completely depleted of lymphocytes.

Plutonium was deposited irregularly in the thymus. Areas containing plutonium were depleted and adjacent uncontaminated areas were relatively normal.

[1] Work done under contract between University of Chicago and Manhattan District, Corps of Engineers, War Department.

32. Structural aspects of inhibitory and facilitatory reticulospinal connections. W. T. NIEMER and H. W. MAGOUN, Department of Anatomy, Northwestern University Medical School.

Information concerning reticulo-spinal connections has been obtained by exciting brain stem components recently found to exert inhibitory or facilitatory influences upon spinal motor activity.

Inhibition of cortically or reflexly induced movement, elicited from the ventromedial bulbar reticular formation, results from activating intrinsically bulbar elements for it is obtained unimpaired after chronic hemisection of the midbrain or pons. Reticulo-spinal connections mediating inhibition descend through the ventrolateral portion of the cord, being most concentrated in the ventral third of the lateral funiculus. Though their influence is predominantly ipsilateral, some spinal crossing occurs.

Facilitation of movement is obtained by exciting the basal diencephalon, midbrain and pontile tegmentum and bulbar reticular formation, outside the inhibitory field. Facilitatory effects elicited from the tegmentum below chronic hemisections of the midbrain or pons indicate the existence of intrinsic facilitatory components at successive levels down the brain stem. Bilateral facilitation of movement is evoked from one side of the brain stem, the crossed effect involving both brain

stem and spinal decussations. In the cord, facilitatory reticulo-spinal connections overlap those mediating inhibition but in general are more dorsally situated, being most concentrated in the middle third of the lateral funiculus.

In spite of their overlap in the cord, facilitatory and inhibitory paths possess sufficiently distinct concentrations to permit the selective elimination of either facilitation or inhibition by appropriately placed spinal lesions.

3. Histochemical comparison of the "resting" and "exhausted" cells of the pancreas and the salivary glands of the mouse and rat. Charles R. NOBACK, Department of Anatomy, Long Island College of Medicine.

The "resting" pancreatic acinar cell (apical zone filled with zymogen granules) shows strong basophilia in the basal zone, strong lipase activity in the apical zone and slight alkaline and acid phosphatase activities throughout the cytoplasm. Lipase activity in the basal zone was noted but may be due to the limitation of the technique.

The "exhausted" acinar cell (induced by pilocarpine) shows more intense basophilia in the basal zone than in the "resting" cell. Traces of basophilia were identified apically. All basophilia, identified as ribose nucleic acid, was abolished in all acinar cells by ribonuclease. Either slight or no lipase activity was observed in the cells. This loss of lipase activity was corroborated by chemical determinations. No changes were noted in the activity of phosphatases.

Basophilia in "resting" and exhausted submaxillary and parotid gland will be discussed.

Phosphatases are abundant in the submaxillary gland, less abundant in the parotid gland, present in slight amounts in the pancreas and practically absent in the sublingual glands. Alkaline phosphatase activity is most intense in sections incubated at pH 8 in the submaxillary gland and at pH 8.5 in the parotid gland. In the submaxillary glands "serozymogenic" cells contain more of the phosphatases than "special serous" cells.

125. The effects of exposure to beta rays on the cerebral cortex of the cat. Rosalind NOVICK, Department of Anatomy, University of Minnesota Medical School. (Introduced by Fred Kolouch.)

Changes induced in the cerebral cortex by exposure to beta rays have been investigated in a series of cats. In these animals capillary tubes of radium emanation were applied directly to the surface of the cranial dura over the cerebral cortex, to give dosages of 60 to 2200 millicurie minutes. Sharply circumscribed lesions, consisting of concentric zones varying in the severity of the alterations, are produced. From the center outwards are found zones of necrotic tissue, then shrunken and hyperchromic neurons, followed by a thin layer of acutely swollen nerve cells, and, finally, the unaltered brain substance. The most severely injured neurons show an early disintegration of cytoplasm, accompanied by an aggregation of nuclear chromatin into large round clumps, and, ultimately, the disintegration of the nucleus. The astrocytes in the zone of the lesion become swollen and fragmented. The oligodendroglia undergo changes which result in the

appearance of cytoplasm, the rounding-up of the cell, and the progression towards a darker, more closely packed nucleus. Microglia also show similar tendencies, and the 2 cell types become indistinguishable.

27. The morphology and behavior of mast cells obtained from mastocytomas and cultivated in vitro. George H. PAFF, Frank BLOOM, and Christopher REILLY, Departments of Anatomy and Pathology, Long Island College of Medicine.

Mast cells from 2 mast cell tumors of dogs have been cultivated in vitro for several months. Observations were made on living unstained cells kept in roller tubes and in hanging drops; on surviving toluidine blue stained cells; on fixed cells stained with iron hematoxylin. Direct observations were supplemented by time-lapse cinematographic studies.

In some of the roller tube cultures mast cells grew as epithelioid sheets. In hanging drops cells grew as irregular spindle and multi-processed cells. All cells which grew from the original fragment contained cytoplasmic granules which stained metrachromatically with toluidin blue despite the fact that the original tumor contained other cell types. It is suggested that mast cells may have a growth inhibitory function on other cells possibly by elaboration of heparin.

Although many cells were mature as judged by the presence of many large cytoplasmic granules, other cells had granules varying from a dust-like to a coarse size. In many cells there was a perinuclear area devoid of granules.

Cinematographic studies for periods of 24 hours revealed that division occurred in some cells by amitosis, although fixed, stained cultures showed mitotic figures. Ameboid movement occurred in some cells while others in the vicinity failed to retract a process or change shape for as long as 6 hours.

The relationship of these mast cells to heparin formation will be briefly discussed.

110. An x-ray motion picture of the thorax. Paul R. PATEK and Irving REHMAN, Department of Anatomy, The University of Southern California.

The x-ray motion picture illustrates the radiographic anatomy of the living human thorax. These x-ray motion pictures demonstrate the movements of the heart during the resting state and immediately following mild and violent exercise. The excursions of the diaphragm are visualized during shallow, normal, and deep breathing as well as during thoracic and abdominal respiration. The respiratory cycle of the lungs during normal and forced and also during abdominal and thoracic types of respiration are shown.

67. Local overgrowth as a possible factor in embryological malformations of the brain. Bradley M. PATTEN, Department of Anatomy, University of Michigan Medical School.

Evidence suggesting the possibility that local overgrowth of the neural plate in early stages of its development might be a factor in the genesis of myeloschisis and spina bifida was presented at the 1946 Anatomists' meetings. During the

past year extension of this study to cover the other parts of the central nervous system has brought to light several instances of disturbances in brain development in which local overgrowth appears to be an underlying factor. Photomicrographs are presented showing examples of such areas of abnormal growth from specimens in the Michigan collection. In these embryos, ranging from 7.5 to 30 mm, local portions of the walls of the neural tube are richly plicated as if they had grown too large for the space in the head which they were destined to occupy. In some cases the redundant brain walls have caused the head to become grotesquely high-crowned; in others the overgrown brain protrudes as an encephalocoele. A preliminary survey of the Carnegie collection shows the availability there of similar cases with which the series can be extended. These findings suggest that a causative mechanism entirely different from an "arrest of development" may lie behind certain types of embryological disturbances. If this concept is borne out it would indicate the desirability of giving increased attention to growth accelerating factors as possible causative agents in abnormal growth.

36. Crossed fibers in the facial nerve in human embryos and fetuses. Anthony A. PEARSON, Department of Anatomy, University of Oregon Medical School, Portland, Oregon.

At the pontine flexure of the brain stem there is a layer of fibers just beneath the floor of the fourth ventricle. These fibers pass toward the midline and many of them cross to the opposite side. The rostral part of this layer includes fibers of the trigeminal nerve. Among the more caudal fibers of this layer are fibers of the facial nerve.

Only part of the motor fibers of the facial nerve follow the course of its internal genu. There are fibers from the rostral end of the facial nucleus which pass dorsally to enter the root of VII without taking the course around the abducens nucleus. Some of these fibers continue toward the midline and are thought to join the facial nerve on the opposite side. This is more clearly shown in the brain stem of an anencephalic baby. In this specimen the facial nucleus is divided into 2 main groups. A caudal part sends its axons through the path of the internal genu. Fibers from the rostral part of the nucleus enter the facial root more directly. Many of them pass toward the midline and are thought to cross the raphe.

Some of the fibers of nervus intermedius enter the fasciculus solitarius, and others continue toward the midline and join the layer of fibers beneath the floor of the fourth ventricle.

73. A study of the factors controlling the differentiation of Mauthner's cell in Amblystoma. Jean PIATT, Department of Anatomy, University of Pennsylvania.

Mauthner's cell in Amblystoma occurs at the level of the VIIIth nerve roots and is in close anatomical and functional relationship with these roots, also the VIIth and Xth lateral-line roots. The consistent association between this giant neuron and the vestibular and lateral-line roots suggests the possibility that this relationship may be one of cause and effect, i.e., Mauthner's cell may differentiate

under the influence of VIIIth or lateral-line nerve roots. A greatly varied series of experiments was carried out on Amblystoma punctatum embryos to test this thesis.

Mauthner's cell failed to differentiate in about one-third of those cases in which the VIIIth roots were prevented from developing. A supernumerary Mauthner's cell developed in a few cases in which experimentally induced ectopic VIIth, Xth lateral-line and VIIIth roots entered the brain at heterotopic levels, more frequently in association with the VIIIth. Neither consistent nor positive results accrued in many cases but the data, on the whole, indicate that the differentiation of Mauthner's cell is influenced to some extent by the presence of VIIIth, chiefly, and perhaps also VIIth and Xth lateral-line nerve roots. A total of 212 experimental animals were individually studied and form the basis for these tentative conclusions.

38. Myelinated fibers in grey rami communicantes. Joseph PICK, Department of Anatomy, New York University College of Medicine.

The source and direction of fine myelinated fibers in grey rami communicantes to the fifth, sixth, seventh lumbar and first sacral spinal nerves were studied by secondary degeneration in 17 cats on osmic acid preparations. The lumbo-sacral sympathetic of 12 sides in normal cats were used as material for comparison. Four series of experiments were performed. Series I included simple division of the sympathetic trunk or isolation of the sixth lumbar sympathetic ganglion by sectioning its internodal connections. Series II consisted in removing the spinal roots and dorsal rootganglia of the nerves to the lumbo-sacral plexus. Series III was a combined operation as performed in series I and II. In series IV the trunk was cut at the second lumbar segment followed by the removal of the spinal roots and dorsal rootganglia of the first lumbar to the second sacral nerves.

In none of these experiments was there apparent degeneration of the main bulk of myelinated fibers (measuring 2–6 μ) within the grey rami. The majority of these myelinated elements were interpreted as postganglionic to the lumbo-sacral plexus. The few large myelinated fibers (measuring 10–12 μ) occasionally seen in the lumbo-sacral sympathetic were apparently somatic to the psoas muscle and to the ligaments of the spine.

59. Effects of experimental cryptorchidism upon the thymus and mediastinal lymph nodes in the rat. James C. PLAGGE, Department of Anatomy, University of Illinois, College of Medicine.

Abdominal confinement of the testes of the rat from the fiftieth to the eightieth day of life did not increase significantly the weights of the thymus or mediastinal lymph nodes. Observations on the seminal vesicles, coagulating glands and ventral prostates of these animals showed that only the latter were significantly lower in weight.

A marked increase in the weights of the thymus and the lymph nodes was noted in male rats after castration for a corresponding 30-day period.

Body weight was definitely decreased by castration but not by cryptorchidism.

41. The vascular pattern of the endometrium of the pregnant rhesus monkey. Elizabeth M. RAMSEY, Carnegie Embryological Laboratory. (Introduced by Robert K. Burns.)

A series of injected maternal placentas shows that connection between the intervillous space (IVS) and the spiral arteries is established about the eighteenth day. Continued arterial growth and progressive narrowing of the endometrium by pressure of the conceptus produce increased coiling and back and forth looping of the arteries. The number of arteries communicating with the IVS is increased by development of small branches into independent stems. Subsequently the ends of adjacent vessels coalesce to enter by a common mouthpiece and finally the number of communications is again reduced by obliteration of many vessels through occlusive intimal proliferation. In the final trimester, as the uterine wall stretches, the coils of the arteries are "paid out" and their course becomes circumferential and gently undulating. Endothelial proliferation early appears in the terminal portion of the artery, increasing in amount and proximal extent until at about day 50 it reaches nearly to the muscularis; thereafter diminishing. The terminal mouthpiece lacks a continuous media and adventitia; is lax and distended. The distal portion maintains its tone.

The veins draining the IVS decrease in number and become greatly distended. Exit from the IVS occurs in later stages only at the margins of cotyledons and at the placental margin.

A separate, relatively inconspicuous vascular system which retains most of its preimplantation characteristics supplies the endometrial wall per se.

34. The course of the fibers from nucleus cuneatus and nucleus gracilis through the human brain stem. A. T. RASMUSSEN, Department of Anatomy and W. T. PEYTON, Department of Surgery, University of Minnesota.

Swank-Davenport preparations of the entire brain stem and thalamus of a 41-year-old man that died 9 days after surgical tractotomy show the following features that are at variance with the generally accepted notion concerning this tract: looping of internal arcuate fibers (in narrow sense) around the solitary fasciculus; invasion of the region of the tectospinal fasciculus, extending the dorsal limits of the medial lemniscus; absence of degeneration in the ventral external arcuate fibers; invasion of the ventral two-thirds of the lateral lemniscus throughout the midbrain. Termination in a restricted region of the posterolateral ventral nucleus of the thalamus conforms to experimental work on higher animals.

42. Influence of shape and size of a conceptus on maternal blood-flow through the uterus of the rabbit: vascular readjustments during uterine accommodation. S. R. M. REYNOLDS, Department of Embryology, Carnegie Institution of Washington.

The shape of the rabbit conceptus is spheroidal after implantation. The spheroid increases in size until the twenty-second day, whereupon it undergoes rapid conversion to a cylindrical form. Measurements of the rate of maternal blood-flow about the spheroidal conceptus show that there is progressively im-

paired flow of maternal blood through the lateral collecting vein in the uterine wall. Upon attainment of maximum spheroidal size there is sudden and profound ischemia. After conversion to the cylindrical shape, there is a quick restoration of the local blood-flow about the conceptus. This is maintained throughout most of the period of rapid fetal growth.

Injection of a diffusible dye through the vascular tree under 110 mm of mercury pressure reveals an inability of the dye to reach the uterine wall in appreciable concentration about the conceptus at the time of local ischemia. Injection of the venous system of the uterus with vinylite and digesting away the tissues shows that during the phase of spheroid shape of the conceptus the collecting veins about the conceptus are placed under increasing tension and are widely separated. This condition is most pronounced at the time of uterine ischemia. After conversion, the collecting veins return toward each other, as some of the veins which were crowded into interconceptus sites take a new position over the growing fetus.

47. The effect of sulfaguanidine on reproduction in the rat. Philip V. ROGERS, Department of Biology, Hamilton College.

A group of young female rats and the males known to have sired their previous litters received a diet containing 2.5% sulfaguanidine. About 30 days later 2 of the animals were discarded because of abnormal estrous cycles. Lack of sperm in the vaginal smears and the continuance of normal cycles indicated that 3 of the remaining 23 rats failed to copulate when placed in separate cages with the males for several estrous periods. An additional 3 copulated but failed to conceive. The others became pregnant and had an average of 3.2 offspring compared with 9.9 in the litters born before they had received the sulfa drug. Six of these produced no visible offspring even though post midterm bleeding was observed in each case.

A second group of animals was given sulfaguanidine and mated in the same manner. A laparotomy was performed on each female 8 days after pregnancy started. One animal showed no implantation sites. With minor exceptions the number of offspring born to the remaining 7 corresponded exactly with the number of embryos observed during the exploratory operation. The average litter of the group was 3.2. Virtually the same results were obtained on twenty second-generation sulfa rats. Laparotomy on the 10 females of this group revealed an average of 3.3 embryos. With 1 exception all were born.

9. Changes in the human skull associated with muscle atrophy resulting from anterior poliomyelitis and other causes. William M. ROGERS, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

Alterations in the form of certain bones, subsequent to atrophy of muscles innervated by the fifth cranial nerve, have been observed in 6 patients and 1 cadaver.

These studies included 3 cases of anterior poliomyelitis, 1 of atrophy of masseter, internal pterygoid and temporalis, 1 of atrophy of masseter associated with

scleroderma, 1 of atrophy of masseter and of both pterygoids with hypertrophy of the temporalis, and 1 adult cadaver.

In each case the atrophy was unilateral with marked asymmetry of the face.

Conventional roentgen films and laminagrams showed appreciable reduction in size of those parts of bones to which atrophic muscles attached.

In 7 cases of atrophy of masseter and internal pterygoid, reduction of bone at the angle of the mandible and a decrease in the vertical dimension of the zygomatic arch occurred. In 4 cases atrophy of the temporalis presented a smaller coronoid process and indistinct temporal lines varying with degree of muscle atrophy.

In 5 cases regressive changes in the condyle were associated with decreased function of the external pterygoid.

On the opposite side of the head, there was a compensatory hypertrophy of muscles and of those parts of bones to which these enlarged muscles were attached. The increased function of the hypertrophic muscles also caused an enlargement of the condyle.

121. Retardation of development in the kidneys of mentally deficient patients. Edward C. ROOSEN-RUNGE, Department of Anatomy, University of Louisville.

The kidneys of patients with mental deficiency (99 cases of prenatal or natal origin) were found to be underweight in about 70% of the cases; below 12 years of age 85% were underweight. The kidneys were small even in relation to the relatively low body length of the patients and the smallness could not be explained by shrinkage following pathological lesions.

Histological examinations revealed a retardation in development on the basis of the following criteria: (1) The histological and cellular differentiation of the glomeruli was often delayed. (2) The average diameter of the glomeruli increased at a retarded rate and rarely reached normal values in the adult. (3) The uriniferous tubules remained below normal in diameter and did not expand in length at the normal rate as indicated by the relatively poor development of the cortex and particularly of the "cortex corticis" (Hyrtl).

It was concluded that damage to the central nervous system which occurs early in life, before or around the time of birth, almost invariably causes a retardation of differentiation and growth of the kidneys. In later life, the underdeveloped kidneys become very susceptible to disease which most often takes the form of nephrosclerosis.

127. Changes in islets of Langerhans in living mice after alloxan administration. Chanai RUANGSIRI, Department of Anatomy, Washington University. (Introduced by E. V. Cowdry.)

Approximately 100 young Swiss mice were studied at intervals after intraperitoneal injection with alloxan. The pancreas was exposed by Covell's method ('28) and then stained with neutral red and janus green in normal saline solution.

Less than 30 minutes after alloxan injection, B cells formed clefts of fluid in cytoplasm, which gradually enlarged and fused with fluids in neighboring

cells. Later, the fluid escaped from the cells into the intercellular spaces and accumulated at pericapillary spaces. Fluid gradually drained into capillaries.

The B granules disappeared where fluid was accumulating. Around the fluid clefts in the cytoplasm, the granules formed clumps. Elsewhere they appeared normal.

The nuclei decreased in size in about an hour, later became distorted. At 8–9 hours they appeared to crack and fragment. After 10 hours, only fragments of nuclei were seen.

Contrary to general belief, A cells also showed signs of degeneration by degranulation, vacuolation and disintegration of cells.

In alloxan-treated animals, endothelial cells of the capillaries were swollen. Leukocytes stuck to capillary walls and capillaries were blocked off.

One hour after alloxan injection, increase in wandering cells was generalized. Later, islets were infiltrated with wandering cells and macrophages. Mitoses were commonly seen both in macrophages and mononuclear wandering cells.

119. The vascular channels of human bone. Elbert B. RUTH, Department of Anatomy, University of Cincinnati.

The vascular channels of human bone are described, from early fetal to late adult life, as seen by the Colquhoun mercuric sulfide and the Ruth thick section techniques. On the basis of this study it is suggested that the vascular channels be designated as pericortical, with reference to the channels of vessels grooving the external surface of the cortex and giving rise to the vessels that enter the bone through the external canals of Volkmann; and endocortical, with reference to all of the canals and canaliculi found in the cortex of compact bone, and including the following: nutrient (medullary) canal, external canals of Volkmann, internal canals of Volkmann, Haversian canals, inter-Haversian canals, translamellar canals, longitudinal interlamellar canals, circumferential interlamellar canals, Haversian canaliculi, and lacunar canaliculi. These channels, with their relationships to each other, and to the bone as a whole, are described at different ages from the 3 months fetus to the age of 93 years — with special reference to the bone picture from birth to maturity. It is suggested that these vascular channels of bone form an hydrokinetic system which offers an explanation of various phenomena of bone development and physiology.

72. Cholinergic drugs and the hypophysis in salamander larvae. Charles H. SAWYER, Department of Anatomy, Duke University School of Medicine.

Larvae of Amblystoma punctatum reared in solutions of various cholinergic drugs develop certain characteristics in common with Blount's ('32) triple-pituitary animals. For instance, in physostigmine the larvae become intensely dark and their gills and dorsal fins are much reduced. These observations led the author to investigate the cholinergic-hypophyseal relationships, of which only the pigmentation effects are considered in the present report. In the responsiveness of pigment cells to stimulation, 2 developmental stages may be distinguished. In the first, or *pre-pituitary* stage (before the hypophysis has become functional),

dermal melanophores attain maximal expansion directly in response to cholinergic drugs, and atropine induces pigment cell contraction. In the second, or *pituitary* stage, the melanophores give very little or no response to cholinergic drugs directly (in hypophysectomized animals) but expand maximally in intact larvae cultured in acetylcholine, mecholyl, physostigmine or prostigmine. The latter effects have been blocked by neither atropine nor nicotine. The results are interpreted as indicating that secretion of the melanophore-expanding hormone of the hypophysis is stimulated by cholinergic drugs.

15. Combined injection and dissection studies to demonstrate variations of the bronchopulmonary segments of the left upper lobe. J. Gordon SCANNELL, Department of Anatomy, University of Minnesota. (Introduced by E. A. Boyden.)

A series of 13 left upper lobes were studied by a combined injection and dissection technic in order to correlate variations of the surface projections of bronchopulmonary segments with variations of bronchial pattern. Unembalmed, "normal" lungs were used. After preliminary dissection, the various segmental and subsegmental bronchi were differentially injected with colored gelatin in the manner of Brock. After formalin fixation detailed dissection of the bronchi was carried out and camera lucida records made.

Variants of hilar bronchial anatomy were interpreted in the light of recent studies by Boyden and Hartmann. Typical and atypical specimens were selected for demonstration of the following major variants: (1) the development of an accessory anterior bronchus, BX^2b, with downward displacement of the anterior segmental bronchus; (2) such variations in segmental representation along the interlobar fissure as absence of the posterior ramus of the anterior segmental bronchus, B^2a, with the appearance of an accessory bronchus or the compensatory expansion of superior lingular and posterior segments; (3) variations of bronchial supply to the apex.

Attention is directed to 3 characteristic ridges on the medial aspect of the left upper lobe, herein named the subclavian, lingular, and posterior crests.

93. Effects of pamaquine on the central nervous system. Ida G. SCHMIDT, Department of Anatomy, University of Cincinnati.

As reported previously, plasmocid, an 8-aminoquinoline with antimalarial properties, produced a complex neurological syndrome when administered to the rhesus monkey. This syndrome was associated with extreme degenerative lesions in the nuclei of nerves III, IV, VI and VIII, cerebellar nuclei and in most nuclear groups associated with their pathways, and with less severe and extensive lesions in other areas.

Pamaquine, although closely related to plasmocid in chemical structure, affected the heart, circulation, and formed elements of the peripheral blood and bone marrow and gave no outward evidence of inducing central nervous system injury. However, it did produce microscopic lesions in the brain stem. Small doses given over a 21-day period gave rise to mild swelling and chromatolysis and/or moderate pyknosis of limited numbers of cells in the nuclei of nerves III, IV and VI.

Scattered cells in some or all of the following nuclei were similarly affected: supraspinal, ambiguus, lateral reticular, lateral cuneate, dorsal motor, vestibular, cerebellar and large-celled nucleus ruber.

Larger doses given repeatedly produced marked swelling and chromatolysis in the nuclei of nerves III, IV and VI, sometimes in the interstitial nucleus of Cajal, and to a lesser and more variabe extent in the other nuclei mentioned above. These changes were in all respects much less than those induced by plasmocid.

132. Histological studies concerning the endocrine kidney. Hans SELYE and Helen STONE, Institut de Médecine et de Chirurgie expérimentales, Université de Montréal, Montréal, Canada.

The excretory function of 1 kidney has been selectively abolished in the rat, using a method described in a previous communication [Journal of Urology *56*, 399 (1946)], by decreasing the blood pressure to a level at which filtration becomes impossible, but the nutrition of the renal tissue is not seriously affected.

Most animals so treated develop a rapidly fatal hypertension, periarteritis nodosa, cardiac infarcts and sometimes hemorrhages into the brain. However, some of them survive the acute critical phase and then develop a certain degree of resistance to the activity of the purely "endocrine kidney."

The juxta-glomerular apparatus is often excessively developed, but usually, it is not visible in "endocrine kidneys" at the height of the hypertensive reaction.

Methyl-testosterone stimulates epithelial growth, even in "purely endocrine" kidneys, a fact which supports the view that this steroid has a direct renotrophic effect independent of its action upon urine formation.

Bilateral adrenalectomy inhibits the normal, rapid, post-operative decrease in the size of the kidney, and thus interferes with the "endocrine transformation" of the organ.

(This work was performed with the aid of a grant of the Commonwealth Fund.)

84. Gigantism produced in normal female rats by chronic treatment with pure pituitary growth hormone. I. Growth and organ changes. Miriam E. SIMPSON and Choh Hao LI, Institute of Experimental Biology, University of California.

Pure growth hormone caused continuous growth in rats when injected chronically from 211 to 647 days of age. The daily dose was gradually increased from 0.4 to 2.0 mg, administered intraperitoneally. The initial growth rate, 22 gm per 10 days, had slowed to a fifth this rate by the end of the experiment. The average final weight of the 8 injected animals was 550 gm, of the controls, 300 gm. The length of the injected animals exceeded that of the controls by 4.5 cm. The liver, kidneys, heart, stomach and intestine increased in weight in proportion to body weight. Weights of endocrine and reproductive organs bore no relation to body weight. The average absolute weights of ovaries, uteri, preputials, adrenals and pituitaries were slightly increased above those of controls. The weight increases of the adrenals and pituitaries were statistically barely significant; that of the preputials was highly significant. Thyroid weights were slightly decreased. Lobular-alveolar development of the mammary tree occurred and was in contrast with the simpler tree of the controls.

5. *Hyaluronidase and the growth of malignant epithelial tumors.* W. L. SIMPSON and A. R. Gopal AYENGAR, Barnard Free Skin and Cancer Hospital and Department of Anatomy, Washington University School of Medicine.

The association of "spreading factors" with the growth of malignant epithelial tumors has been recognized for some years, although the nature of this relationship has never been elucidated. Suggestions on the possible nature of this association were made by Cramer and Simpson in 1943. The present report describes preliminary experimental efforts to test these hypotheses. The effects of a "spreading factor" from the testis (hyaluronidase) on the growth and invasive capacities of a mouse transplantable squamous cell carcinoma are described, as well as the relationship between the injection of hyaluronidase and the development of malignant skin tumors in response to 20-methylcholanthrene.

Numerous examples of enhancement of invasive growth, with the destruction of muscle and bone, demonstrate the striking effect of the local injection of the hyaluronidase about the transplantable carcinoma. In a few cases distant metastatic lesions have developed very soon after this type of treatment. On the basis of these preliminary experiments further studies have been instituted into the mechanism of this relationship.

23. *The responses of syncytium and fibrinoid of the human placenta to acid and basic dyes under controlled conditions of staining.* Marcus SINGER and George B. WISLOCKI, Department of Anatomy, Harvard Medical School.

Employing a technique of histochemical study in which tissue sections are stained in solutions of varying pH but in which other conditions such as dye concentration, ionic strength and temperature are held constant, we have investigated the staining characteristics of fibrinoid and placental syncytium. When the light absorption of these placental structures, measured by photometric means, is plotted against the pH of staining, curves are obtained which characterize the constituent proteins. The syncytium of the early placenta possesses 3 areas distinguished by their responses to acid and basic dyes: (1) an outer brush border, having a low affinity for both of these dyes; (2) an outer zone notably acidophilic in character; and (3) an inner or nuclear zone the cytoplasm of which is relatively basophilic. Comparison of the staining indices of placental syncytium at various ages shows that there are well-defined changes in affinity for acid and basic dyes which occur especially during the first few months of development. These changes involve a rapid increase in the capacity to bind acid dyes and a gradual decrease in affinity for basic dyes.

The staining characteristics of placental fibrin and fibrinoid are similar to one another and show little variation with age. The staining indices of both resemble closely that of a sectioned plasma clot.

79. *Mitosis and regeneration in the corneal epithelium of rats as affected by hypo- and hyperthyroidism.* George K. SMELSER, Columbia University.

The rate of epithelization of standard thermal burns of the rat cornea was determined in thyroidectomized, in normal and in hyperthyroid rats. The burns of the thyroidectomized animals healed at a normal rate, but those of the hyper-

thyroid were slower in healing. The number of mitotic figures in the regenerating epithelium was significantly greater than that in the intact cornea of the left eye in 62.5% of normal and 83% of the thyroidectomized rats but in only 37.5% of the hyperthyroid group.

The number of mitotic figures in the intact corneal epithelium was the same in normal, hypo- and hyperthyroid rats, however, in colchicine treated rats the number found in the hyperthyroid animals was 40% less than that in control corneas. Colchicine effectively arrested mitotic division in metaphase in all rats.

The data suggest that excess of thyroid hormone reduces the speed of cell movement in regenerating corneal epithelium and lengthens the time required in a mitotic division. Ablation of the thyroid, resulting in serious impairment of body growth, did not interfere with these processes.

75. The adrenal glands of normal and castrated mice. Fern W. SMITH, Department of Anatomy, Yale University. (Introduced by William U. Gardner.)

The lipid content of adrenal cortical cells in mature (10–12 mo.) male and female mice of several inbred strains (A, JK, L, CHI, I and N) and hybrids (A × C_3H and NHO) were studied. Frozen and paraffin sections of formalin and Bouin's fixed tissue were examined when stained with haematoxylin and eosin, Sudan IV and Sudan Black B. Additional sections were studied following the Schultz test for cholesterol and with polarized light. Hyperplasia of the adrenal cortex occurs subsequent to castration (Woolley, '39; Smith, '45). These hyperplastic areas in the adrenal cortices of castrated mice, studied by the same methods, were compared with the normal adrenal glands. The Sudan stains indicated a concentration of lipids in the outer zona fasciculata of intact animals. The extent of involvement of the zona glomerulosa and juxta-medullary areas was variable. In the castrated animals the distribution of lipids in the fascicular layers was similar to that found in control mice of the same strain. In the hyperplastic areas of the cortex in the castrates, the enlarged, vesicular, lightly staining cells (haem. and eosin) were observed to be highly sudanophilic, and to contain birefringent globules and cholesterol. Dustlike sudanophilic particles were found in the small round subcapsular type of cell but consistent results have not been obtained with polarized light or with the Schultz test.

30. The relation between structure and function in the cerebral cortex.[1] Wilbur K. SMITH, Department of Anatomy, University of Rochester, School of Medicine and Dentistry.

Largely as a result of studies on the precentral cortex in primates and the corresponding cortical region in other mammals, there has arisen the general concept that a specific relation exists between cytoarchitecture and response elicited by electrical excitation. Evidence against this view is furnished by studies on the frontal eye fields, the cingular cortex and the pyriform lobe.

In the frontal cortex the electrically excitable region yielding ocular responses is not limited to a single cytoarchitectural area. Furthermore, within the eye fields responses similar in character have been obtained from cortex differing in neuronal arrangement.

The rostral cingular cortex, designated as area 24 by Brodmann from studies on Cercopithecus, is a unique region of the cortex in that it yields a number of divergent responses. If this cortical region possessed cytoarchitectural uniformity, it would offer substantial evidence against the above concept. However, the cortex included by Brodmann in area 24 varys so widely in cellular arrangements that it can hardly be considered as constituting a single area. Studies on this region have thus far failed to yield a constant relation between the structure of the responsive focus and the response elicited.

Investigations on the pyriform lobe and on other parts of the cerebral cortex yield evidence supporting the view that the responses elicited depend mostly, perhaps entirely, upon the connections of the cortical cells with lower levels rather than upon cytoarchitecture.

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[1] Aided by a grant from The John and Mary R. Markle Foundation.

117. Multisinusal versus nonsinusal spleens. Theodore SNOOK, Department of Anatomy, Tulane University.

It was recently suggested (MacKenzie et al., '41) that the term ''sinus'' (venous sinus) is not an apt designation for the primary venules in the splenic pulp of mice, rats, rabbits, guinea pigs and cats. ''Capillary veins'' or ''primordial veins'' were favored as more applicable terms.

Examination of sections of spleens (human, monkey, guinea pig, dog, rabbit, rat, squirrel, skunk, cat, cow, horse, pig, mole, bat, weasel) impregnated for reticulum has shown that the suggestions given above hold for the spleens of the cat, cow, horse, pig, mole, bat and weasel. Here sinuses are lacking, and the finest venous tributaries are continuous with the pulp reticular spaces. However, in the spleens of the other animals listed (e.g., human, monkey, guinea pig, dog, rabbit, rat, squirrel, skunk) true venous sinuses (Milzsinusen) with characteristic lining cells and reticular fibrous support are present and occupy a large portion of the red pulp. The course followed by arterial blood to the sinuses appears to be distinctive for each animal studied.

It seems desirable to classify mammalian spleens in 2 groups: those whose spleens are multisinusal; and those which are nonsinusal.

89. Reinnervation phenomena as revealed by prolonged observations of vagus nerve stumps and associated sense organs. Carl Caskey SPEIDEL, School of Anatomy, University of Virginia.

Multiple successive operations on vagus nerves in frog tadpoles have yielded clearcut data on trophic and tropic effects. Individual nerve stumps and associated lateral-line sense organs have been kept under observation for many months.

Compensatory reinnervation progresses readily along distal stumps of freshly cut nerves but not along old distal stumps. Loop or plexus formation between vagus branches of right and left sides sometimes occurs. In such tadpoles, a whole zone of sense organs may be alternately shifted experimentally from an innervation by fibers of one side to those of the other, then back again, etc. An interesting variety of reversed (distal to proximal) innervation is also possible.

All stages in individual sense organ atrophy and degeneration, together with recovery, have been watched, and correlated with periods of denervation and reinnervation. Complete degeneration and failure of recovery have also been noted. Sense organs are not induced by nerves from indifferent epithelium. Denervated organs of a few months' standing have less capacity for regeneration and readjustment than freshly denervated or innervated organs.

Frustrated nerves without organs, as well as organs without nerves, waste away and die. Stranded sheath cells on aneural stumps atrophy and ultimately disappear. Old aneural stumps of more than 6 weeks' standing display marked loss of ability to penetrate repair zones as compared with fresh distal stumps.

4. *Histological studies on nerve elements and their endings at the epithelial cells of the gastric mucosa.* Rosette SPOERRI, Department of Biology, Graduate School of Arts and Science, New York University. (Introduced by Harry A. Charipper.)

The presence of "interstitial neurons" in the lamina propria of the gastric mucosa in guinea pig, cat and man, and their neural nature have been demonstrated using a tribasic staining method and checked by silver techniques. The most significant features in favor of the neural character of these cells are their occurrence as isolated morphological units, their impregnation by silver methods, the presence of a nucleolus in a centrally placed, clear, spherical or ovoid nucleus, of occasional neurofibrils and chromophil substance, the varicose appearance of some processes, and the manner of their termination. The protoplasmic extensions have been observed establishing connections with all types of gastric epithelial cells reaching not only the gastric glands but also the surface epithelium; their terminations could be demonstrated as bulb-like, knob-like and club-like structures or arborizations and could be traced directly to an epithelial cell, sometimes penetrating the glandular epithelium or the surface epithelium thus establishing a closest contact with the epithelial cells themselves. The existence of the interstitial neurons gives a new value to physiological experiments which demonstrate a nervous control of gastric secretion; they may represent an important constitutent and terminal link of the enteric nervous system. The gastro-intestinal innervation thereby may be effected through 3 systems: (1) through the sympathetic and parasympathetic systems; (2) through the intramural ganglion-cell apparatus; and (3) through the interstitial neurons.

132a. *The inductive effect of the intact posterior chorda-mesodermal axis on competent prospective ectoderm in Amblystoma.* Walter R. SPOFFORD, Department of Anatomy, Vanderbilt University.

While it is generally agreed that the neural plate in the salamander originates by means of an inductive event mediated by the underlying chorda-mesoderm, it is by no means apparent from inspection whether the mesoderm arising in the posterior part of the neural plate is the result of the "neural" induction, or whether, like the orthodox mesoderm with which it is continuous around the dorsal lip of the blastopore, it arises by an autogenous self-differentiative process. To determine the nature of the inductive influence of that part of the chorda-mesodermal axis

underlying the posterior neural plate mesoderm, the posterior part of the neural plate was removed from 12 neurulae, and replaced by vitally stained competent prospective ectoderm from stage 11 gastrulae. After delayed closure of the posterior neural folds, the graft material was later found to be disposed as somites in the variously deformed tails of the hosts.

In a second series wherein a larger part of the neural plate was removed and replaced by competent test material, each graft formed spinal cord in its anterior part, and somites in its posterior part.

Although it is well known that there are "regional" differences in the "organizer," this experiment demonstrates a sharp break in the inductive properties of the chorda-mesodermal axis in its posterior region, with neural-inducing capacity anteriorly, and mesodermal-inducting posteriorly.

87. Localization of motor cells in the thoracic spinal cord of the rhesus monkey. James M. SPRAGUE, Department of Anatomy, Johns Hopkins University.

It was shown by the author that the motor cells of the dorsal primary rami (axial muscles), and ventral primary rami (limb muscles) are not segregated into medial and lateral nuclei in the brachial cord of the fetal sheep (J. Comp. Neur., *85:* 127–140). The cells are indiscriminately mixed and show no indication of the localization frequently described in adult mammals. The following report concerns sub-adult rhesus monkeys, in which ventral rami were cut on one side of the animal, and dorsal rami of the same segments on the other. $T_{7, 8, 9}$ were cut in this manner in 1 animal, and $T_{10, 11, 12}$, L_1 in another. In a third both rami were cut on the same side in $T_{5, 6, 7, 8, 9}$. The chromatolytic cells were plotted by means of a calibrated ocular grid. No medial and lateral columns are revealed. Motor cells of both dorsal and ventral rami are distributed throughout the ventral horn. The cells of the dorsal rami extend at least one full segment above and below their nerves of exit. At the upper and lower ends of this distribution the cells are limited to the anterior tip of the ventral horn. In the segments of which the nerves were cut, these cells spread throughout the horn. Ventral rami cells are mostly restricted to the levels of their nerves of exit. They are found everywhere but the anterior tip of the horn.

114. Rate of renewal of the cells of the intestinal epithelium in the rat. C. E. STEVENS and C. P. LEBLOND, Department of Anatomy, McGill University.

The rate of renewal of cells may be estimated by counting the percentage of cells undergoing mitosis. Mitotic counts carried out on the ileum of the rat revealed that the half life of the cells of the epithelium is quite short, approximately 1 to 2 days. In the duodenum, the half life of the epithelial cells is somewhat longer.

The cells of the intestinal epithelium originate from mitoses taking place throughout the length of the Lieberkuhn glands. The cells are then gradually pushed up along the sides of the villi and seem to be eliminated at the tip of the villi where a characteristic structure formed by the dying cells is usually visible.

97. Primitive bloodvessels of the spinal medulla of the rabbit injected while alive. Leon H. STRONG, Department of Anatomy, University of Michigan.

The vascularization of the dorsal and ventral halves of the spinal cord is enough different to suggest a functional significance. The medial ramus of the intersegmental artery forks into a ventral and lateral radical for the ventral and dorsolateral surfaces. The lateral radical ascends to the upper limit of the segmental ganglion, forks caudally and rostrally establishing a longitudinal trunk by anastomosis, but continues its dorsal course. At the ganglion, it sends medially, perpendicular to the lateral surface, 2 parallel capillaries which reach the ependyma. One turns dorsally, the other ventrally, subependymally. The upper capillary branches dorsally about its midpoint. This branch reaches nearly to the surface, parallel to the subependymal capillary before it anastomoses medially with it. Between the 2 dorsal capillaries, transverse parallel capillaries develop, forming a dense subependymal plexus that fills the area between them and connects intersegmentally. The lateral half of the dorsal area of the cord is left nonvascular.

The ventral radical reaches just lateral to the midline ventrally, forks caudally and rostrally, establishing a longitudinal trunk close to its fellow but nonanastomotic with it. From this trunk a subependymal capillary arises dorsally which joins the lower of the 2 medial capillaries from the lateral longitudinal trunk. From the quadrangle of vessels thus formed, long centrally directed capillaries form a central loose plexus.

78. The factor of nutritional interruption in the transplantation of adrenal medullae in the mouse. C. Donnell TURNER, Department of Zoology, Northwestern University.

Unlike the cells of the adrenal cortex, those of the medulla apparently continue their secretory capacity for an indefinite length of time and there is no provision for their replacement. Adrenal medullae persist more frequently in the mouse, when whole glands are transplanted, than in other mammals studied; this is believed to be due to the fact that the medulla of the mouse touches the capsule at the hilus and hence is not completely surrounded by cortical tissue. This makes it possible for nutritional materials of the host to reach the medulla directly without first diffusing through the cortex.

When whole adrenals from young donors are inserted below the kidney capsule of appropriate hosts, the persistence of medullae is correlated with the position of the hilus with reference to the kidney capsule. If the hilus is caused to project through a pore in the kidney capsule, medullae persist in approximately 2% of the cases; medullae persist in about 80% of the grafts when the hilus is firmly pressed into the substance of the kidney cortex, or when whole glands are introduced into the anterior chamber of the eye. The incidence of medullary persistence is increased by sectioning the donor adrenals so as to expose a greater medullary surface. It is concluded that nutritional interruption is an important factor in determining the survival of medullary cells in adrenal grafts.

71. Vascular changes in the gonopodium of poeciliid fishes during the metamorphosis of the anal fin. C. L. TURNER, Department of Zoology, Northwestern University.

In the males of poeciliid fishes various structures, including some of the fins, become profoundly modified in the male with the onset of sexual maturity. Experiments indicate clearly that the modifications of these target organs occur only under the influence of hormone stimulation while other parts of the body are not affected. The same modifications can be induced in females by hormone treatment.

During the process of metamorphosis in the anal fin of the normal males or in females treated with androgenic hormones the vascular systems of the susceptible tissues undergo changes which resemble those of the early stages of inflammation. The changes are marked by the following stages: (1) Dilatation of capillaries and venules and changes in the permeability of the endothelium occur. Leakage of fluid from the blood produces edema in the surrounding tissues. (2) The flow of the blood becomes sluggish. The endothelium becomes sticky and traps masses of blood cells. (3) Stasis occurs and capillaries and venules become blockaded by plugs of blood cells. (4) The endothelium breaks down and the masses of blood cells are digested by fibrocytes or free macrophages. (5) Circulation is reestablished in affected tissues by the formation of new blood vessels.

Ethynyl testosterone in a concentration of 1 mg/20,000,000 cm^3 of water is sufficient to bring about vascular changes in the newly regenerated anal fin of a female.

61. Colchicine influence during embryonic development in rats. John H. VAN DYKE and M. G. RITCHEY, Department of Anatomy, Washington University School of Medicine.

Embryos of pregnant Wistar rats given a single intraperitoneal dose of 0.06 mg of colchicine per 100 gm of body weight on the sixth, eighth, tenth, twelfth or fourteenth day of gestation and sacrificed *3 days later* have been studied.

Embryos thus subjected on day 6 and 8 were dead and partially resorbed. Embryos of females treated on day 10–14 were living but showed general developmental retardation. Three embryos (10 day series) possessed *gross abnormalities.* Two of these specimens developed a transverse crease on the dorsal surface near the arm pads, apparently representing transient growth inhibition resulting from colchicine. Diencephalic hydrocephalus occurred also in one, while the cephalic portion of the other, although enlarged, appeared normal. Below the level of this crease the embryos were bulbous and *necrotic.* The site of maximal mitoses (using neural tube as an index), as well as of aberrant mitotic figures (typical colchicine effect), occurred at the cephalic end of these specimens. A kinky tail characterized a third embryo.

Our initial study suggests that colchicine retards development of advanced embryos but has a lethal effect on young ones. While its influence lacks uniformity, colchicine may temporarily inhibit development, or cause death of a portion differentiating subsequent to its injection (caudal part), but the precocious region (cephalic end) appears more resistant and may survive for a limited period.

10. The relation of the temporal muscle to the form of the skull. S. L. WASHBURN, Anatomy Department, Columbia University.

The effect of the temporal muscle on the developing skull has been debated for many years. In order to determine the relation of the muscle to the form of the skull, unilateral removal of the temporal muscle was performed on day-old rats. In 10 of 22 animals the long neck muscles of the same side were separated from the nuchal crest. The animals were sacrificed from 3 to 5 months later. On the operated side no temporal line formed, the coronoid process of the mandible failed to develop, and the nuchal crest was absent when both temporalis and the nuchal muscles had been removed. The brain case was normal in shape when temporalis alone was absent. After the double operation, a change in internal shape, as shown by endocasts, was discernible. Although the temporal muscle of the rat is large relative to the brain case, effects of its removal are limited to bony crests and processes immediately associated with its origin and insertion. Even after the additional removal of muscles from the nuchal crest, only very minor changes in the form of the endocast can be detected.

76. Studies of regenerating adrenal cortex in the ears of rats, and the ability of such grafts to maintain life. R. G. WILLIAMS, Department of Anatomy, University of Pennsylvania.

Group 1. 28 rats were adrenalectomized. The capsule and zona glomerulosa from 1 gland in each animal were separated as 1 piece and implanted in the left ear; the remainder of the gland was implanted in the right ear.

Group 2. 20 rats were adrenalectomized; 1 gland was split as in the previous group but the pieces were replaced in the original adrenal bed.

Group 3. 10 rats were adrenalectomized and no grafts made.

All animals in Group 3 died within 105 days. Accessory cortical tissue, if present, did not maintain rats in the absence of the chief glands. 10 animals of Group 2 and 12 of Group 1 died within 3 months with symptoms of adrenal deficiency. Grafts at the lower temperature and under the other environmental differences in the ear maintained life as effectively as those in the abdomen. In all animals the original fasciculata and reticularis cells disappeared completely and quickly. Regeneration occurred in all animals but was very limited in those that died. Regeneration was from the glomerulosa, and possibly from the capsule, by centripetal proliferation. Well-defined fasciculata and reticularis were present 20 months after operation. Holocrine secretion appeared to be the fate of some reticularis cells resulting in large spaces which became vascularized by sinusoids.

63. Anomalies of the genito-urinary tract induced by maternal vitamin A deficiency in fetal rats. James G. WILSON, Department of Anatomy, University of Rochester, and Josef WARKANY, Department of Pediatrics, University of Cincinnati.

Fetal rats from mothers maintained in chronic vitamin A deficiency exhibited 2 types of anomalies in the genito-urinary tract. In both sexes epithelia that composed, or were derived from, the urogenital sinus underwent keratinizing metaplasia. Abnormal keratinization first appeared in the urethral plate on the

eighteenth day of gestation and by the twentieth day it was present in all epithelia of sinusal origin distal to the juncture of the genital ducts with the sinus. In older fetuses and newborns keratinization was greater in degree but still largely limited in extent to the genito-urinary tract distal to, and around, the termination of the genital ducts. The metaplastic changes first appeared, and were always more pronounced, on the dorsal wall of both the urogenital sinus and structures derived from it.

Other anomalies consisted of hypoplasias and frank malformations. Relative to development in other regions, the urogenital sinus and its associated structures were retarded. This general retardation was exemplified by such conditions as: retention of the primitive juncture of ureters with the mesonephric ducts; persistence of both sets of genital ducts, Mullerian and mesonephric, in either sex for several days beyond the time regression normally occurs in one set; and failure of the Mullerian ducts to unite distally to form a utero-vaginal canal, even in mature female fetuses. Hypospadias and horseshoe kidneys were commonly occurring malformations.

82. Cytological studies on spontaneously occurring adenomata of the anterior hypophysis of the rat. J. M. WOLFE and A. W. WRIGHT, Departments of Anatomy and Pathology, Albany Medical College.

Three types of spontaneous adenomata have recently been described in the anterior lobes of old female rats; namely, adenomatous nodules, hemorrhagic adenomata and chromophobe adenomata, the last 2 being larger and more discrete than the first (Wolfe, Bryan and Wright, '38). Lesions of the first 2 types often contained acidophiles as well as chromophobes, the latter only chromophobes. Forty-four additional lesions have been studied, including all of the above varieties. Adenomatous cells tended to be enlarged. Enlargement of the nuclei occurred in cells of most lesions while hypertrophy of the nucleoli was found almost constantly. The nuclei were often characterized by wrinkled membranes, lobulation and evidence of nuclear budding; the nucleoli often contained vacuoles. The degree of enlargement of cells, nuclei and nucleoli varied within wide limits and was often extreme. In many adenomatous acidophiles and chromophobes hypertrophy of the Golgi apparatus was pronounced; this was not constant. The mitochondrial picture varied; often they were more numerous than normal; just as frequently only normal numbers were present. They were occasionally quite hypertrophied. The adenomatous acidophiles were invariably packed with granules; there was no cytologic evidence indicating that they were releasing hormone. The cytoplasm of some of the chromophobes was scant and stained light blue; in other cells the cytoplasm appeared abundant, dense and stained deep blue.

105. Changes with age in the weight of the liver in Wistar albino rats.[1] Eleanor H. YEAKEL and Edmond J. FARRIS, The Wistar Institute of Anatomy and Biology.

The weight of the liver was determined in 164 male and female albino rats 21–1000 days of age. The average liver weight increased rapidly from weaning to

75 days, and thereafter maintained a uniform weight to 500 days (approximately 11 grams in males, and 8 grams in females).

In the old age range of 600–900 days, no change in absolute or relative weight of the liver occurred in the male rats, but many of the livers of the females were significantly heavier. At 1000 days, the weight fell in the males, paralleling a loss in body weight, to an average of 9.68 gm. The weight remained high in the females (11.46 gm).

The increase in liver weight in a number of old female rats appeared to be correlated with the presence of tumors elsewhere in the body, specifically in the pituitary, mammary glands, and uterus, rather than with body weight changes. The average weight of the liver in 14 old females with no tumors was 8.91 gm, and in 27 tumor-bearing animals was 12.00 gm. Average relative weights were also greater in the latter group. The weights of mammary gland tumors in 12 old rats compared with the corresponding liver weights suggested that spontaneous mammary growths influenced liver weight when the tumors exceeded a critical weight of approximately 30 gm.

[1] This investigation was aided by a grant from the Samuel S. Fels Fund.

8. Anatomical factors in senile alopecia; experimental production of baldness. M. Wharton YOUNG, Department of Anatomy, Howard University.

The constant pattern of senile alopecia suggests that there is a definite anatomical basis for this condition. The retention of the marginal zone of scalp hair (also other body hair including the beard) indicates that local factors within the scalp are more important etiologically than systemic deficiencies.

The tela subcutanea contains the hair bulbs embedded in fat, also the superficial muscles and the larger vascular networks. Injection studies reveal a richer vascular supply in the marginal (muscular) zone of the scalp than in the central (aponeurotic) zone. Likewise, senile alopecia is confined to the aponeurotic area, whereas, hair persists in the marginal area overlying the occipital and auricular muscles. Consequently, a sharp line of demarcation is established between these 2 contrasting regions. The bald area shows a loss of hair follicles and fat with increased fibrosis in the subcutaneous layer.

Beginning alopecia is associated with tension zones in the scalp resulting in diminished blood supply in these areas. Scalp tension may result from contraction of muscles attached to the galea, from continued growth of the cranium, or from external pressure. Some victims of the atomic explosion developed a "senile-type" of alopecia. Tension areas were produced experimentally in rhesus monkeys by suturing the free scalp edges after excising elliptical segments, thus reducing the circumference. These operations resulted in persistent baldness closely resembling the human types.

46. The ovarian follicular cyst and the pattern of sexual behavior in the female guinea pig. William C. YOUNG, Wesley A. INNES, Richard C. WEBSTER and Paul G. ROOFE, Department of Anatomy, University of Kansas.

In our experience during which the ovaries of several hundred guinea pigs have been examined microscopically we have only recently encountered an animal in which a follicular cyst, to be distinguished from the rete ovarii cyst, was present.

Each ovary was largely medulla partially capped by a relatively inactive cortex. No vesicular and only 15 primary follicles, most undergoing atresia, were found. A hemorrhagic cyst containing strongly eosinophilic luteinized granulosa cells surrounding a degenerating primary oocyte and a corpus luteum-like structure were also present. An enlarged uterus seen only macroscopically was suggestive of pyometra caused by constant estrogenic stimulation; metaplastic epithelium in the rete ovarii was similarly suggestive.

Prior to ovariectomy the animal displayed male-like behavior at cyclic intervals but was not found in heat although after ovariectomy short periods of heat followed the injection of estradiol benzoate and progesterone. The history of the animal considered with other data to be reviewed suggested that the amount of estrogenic stimulation is correlated with the appearance of male-like behavior in female guinea pigs and possibly certain other mammals. In a test of this hypothesis, reduction in the quantity of injected estrogen was followed by a much greater decrease in male-like behavior than in mating responses. The implications for the problem of the physiological and somatic basis of sexual behavior patterns will be discussed.

ABSTRACTS READ BY TITLE

136. The effect of splenectomy and inanition on the bone marrow of plethodontid urodeles. William C. BARRETT, Jr., Department of Anatomy, Western Reserve University.

Splenectomy, either alone or combined with inanition, affords a means of testing the competence of the lymphogranulo-poietic bone marrow of plethodontid salamanders. Fifty-eight specimens of *Desmognathus fuscus* and *Eurycea bislineata* were splenectomized and the blood and bone marrow was studied at intervals for a period of 10 months. Removal of the spleen, the sole erythropoietic organ, did not stimulate erythropoiesis in the bone marrow or in the hepatic or mesenephric hemiopoietic loci. Splenectomy, combined with inanition for 1 to 3 months, and followed by feeding likewise failed to stimulate erythropoiesis. Following simple splenectomy erythropoiesis was confined to the peripheral blood. The number of erythroblasts and hemoblasts in the blood stream increased temporarily, but returned to normal after 4 to 6 weeks. Splenectomy followed by inanition produced a marked anemia and a high mortality rate. Subsequent feeding produced an increase in the hemoblasts and immature erythrocytes accompanied by increased mitotic activity. The bone marrow of the starved and splenectomized animals showed signs of depletion. It is concluded that the bone marrow of plethodontids, normally lymphogranulopoietic, is not a favorable site for erythropoiesis. Also, the spleen, usually considered the amphibian erythropoietic organ, plays a minor role in erythropoiesis compared with the circulating blood. The spleen as well as the lymphogranulopoietic organs contribute undifferentiated elements to the blood where they differentiate heteroplastically into erythroblasts.

137. The mechanism of menstruation. G. W. BARTELMEZ, Department of Anatomy, The University of Chicago.

It has been suggested (Schlegel, '45) that the superficial ischaemia and necrobiosis of menstruation are due, not to constriction of the coiled arteries (Daron, '36) but rather to the opening of arterio-venous anastomoses and the shunting of blood from the surface to such an extent that a superficial ischaemia is produced. If this were true it should be possible to inject particles like starch grains into the arteries and find them in the veins of the endo- and myo-metrium. Certain of our injected macaque uteri provide evidence on this score. They were prepared by filling the entire vascular bed after vasodilatation with carmine gelatin or India ink via the arteries and then injecting starch grains (9–14 μ), which had been colored by reduced silver or glygocen methods. The uteri were serially sectioned at 100–500 μ. One ischaemic (pre-menstrual) case and 5 menstruating uteri (days 1 to 6) were surveyed. Even arteries which contained starch grains in the superficial zone usually showed basal constrictions except in the sixth day case. No starch was found in any vein either in the endometrium or myometrium. It would seem that in the macaque uterine arterio-venous anastomoses are either not patent during menstruation or they are not abundant enough to be significant in the production of the menstrual ischaemia.

138. Stratification of the human erythrocyte by ultracentrifuging. H. W. BEAMS, Department of Zoology, State University of Iowa.

Ultracentrifuged human erythrocytes are stratified into 2 or more layers which may be sharply stained, after fixation in Champy's fluid, by Heidenhain's hematoxylin, or Mallory's triple stain. Such results do not support the earlier views that the interior of the erythrocyte is chiefly of a homogeneous nature. Similar observations have been reported for the erythrocytes of the rat (Beams and Hines, Anat. Rec., *90*).

Many of the leucocytes likewise show a shifting of their components following ultracentrifugation.

139. Evidence for 2 types of thyrotropic activity in Amphibia. Raymond F. BLOUNT and Isabel H. BLOUNT, Department of Anatomy, University of Texas.

The type of activity produced by the thyrotropic hormones of the pituitary at different ages during the initiation of the pituitary function has been studied. Rana pipiens tadpoles were stages within "paddle," premetamorphic and metamorphic groups by the system of Taylor and Kollros ('46). During short intervals within these age brackets animals were placed in thiouracil solution (0.05%) in order that the chemical removal of pituitary inhibition would result in overproduction of the thyrotropic hormone characteristic of each age. Representative test and control animals were prepared for microscopic study. The treated animals showed an increase in the storage of colloid during the premetamorphic stages. The amount of colloid was increased relative to the epithelium. The cells showed no increase in height as compared with the controls such as would be expected if a discharge hormone were present. This phase is of course followed during meta-

morphosis by the production of the thyrotropic hormone causing discharge of colloid. These results support the evidence from additional pituitary transplants in the salamander and from heteroplastic transplantations between a neotenous form, in which storage hormone alone exists, and a metamorphosing form in which a discharge hormone also is present.

140. Vascularization of the cornea of the rat resulting from deficiency of B vitamins. Lester L. BOWLES, Department of Microscopic Anatomy, University of Georgia School of Medicine. (Introduced by Lane H. Allen.)

In the investigation of corneal changes resulting from B vitamin deficiencies, a control diet was used consisting of: "vitamin free" casein 20 gm, cod liver oil 2 gm, cottonseed oil 3 gm, salt mixture 4 gm, sucrose 71 gm, choline chloride 200 mg, calcium pantothenate 2 mg, thiamine chloride 0.4 mg, pyridoxine hydrochloride 0.4 mg and riboflavin 1.6 mg. To produce deficiency of pantothenate, pyridoxine or riboflavin, diets the same as the control were used, but with the respective vitamin omitted.

Nine of 23 rats (6 litters) placed on pyridoxine-deficient diet at 48–57 days of age developed definite corneal vascularization. Six older rats showed no corneal changes, 6 younger rats died too soon for corneal vessels to appear. Thirteen of 22 rats (5 litters) placed on pantothenate-deficient diet at 41–52 days developed extensive corneal vascularization. Five of the 22 and 5 younger rats died too soon for vessels to appear. The corneal capillaries in these deficiencies were numerous and large with prominent anastomoses, as contrasted with the fewer vessels in fine networks resulting from riboflavin deficiency. Correcting the deficiency of pyridoxine, pantothenate or riboflavin in the respective diet resulted in regression of the vascularization.

Litter-mate controls, rats with symptoms of nicotinic acid deficiency, starved rats and rats on a caloric-deficient diet showed no significant corneal changes.

This study was aided by grants from the John and Mary R. Markle Foundation.

141. An analysis of variations in the bronchial pattern of the right upper lobe of 50 lungs. Edward A. BOYDEN, Department of Anatomy, University of Minnesota.

With the cooperation of Dr. J. Gordon Scannell, 50 lobes have been dissected and pictorial records made of the distribution of arteries, veins and bronchi in the 3 bronchopulmonary segments — apical (B^1), anterior (B^2) and posterior (B^3).

In contrast to the left upper lobe, in which the key to variations is the outgrowth from the apical bronchus of an accessory anterior ramus (BX^2b) and the downward displacement of B^2 to form a trifurcate pattern (26%), the striking modification on the right side is the outgrowth from the anterior bronchus of an accessory apical ramus (BX^1b) which takes over the territory usually supplied by B^1b. In half of these 28 cases, not only the whole mediastinal surface of the lobe but also part of the apex is supplied by B^2. Also the prevailing pattern is trifurcate (46%); for although 54% of specimens are bifurcate 10 different combinations of bronchi occur.

Of further interest is the tendency for the clinically important posterior bronchus that borders upon the interlobar fissure, to separate into independent halves; then its upper ramus (B^3a) arises with the apical bronchus, its lower ramus (B^3b) with the anterior bronchus.

Finally, that ramus of the anterior segment which borders on the secondary fissure (B^2a) occurs in 98% of specimens; yet on the left side the corresponding bronchus occurs in only 60% of specimens.

142. Re-growth of nerve fibers in the spinal cord. James O. BROWN, Department of Anatomy, Woman's Medical College of Pennsylvania.

Transected spinal cords in a series of cats and dogs showed evidence of regrowth of nerve fibers. By the end of 24 hours some of the cut fibers already showed signs of regeneration in the cat. These abortive attempts at regeneration appeared as fine fibers growing in various directions. Their disorientation permitted the influx of connective tissue the abundance and rapid growth of which formed a barrier that impeded the slower growing nerve fibers from bridging the gap. This is suggestive of the abortive regeneration in peripheral nerves observed by Perroncito ('07).

Strips of omentum, gall bladder, amnion and aorta were used to wrap around the region of transection in an attempt at preventing the invasion of connective tissue from the dura. None was successful. This corresponds to the already observed interference in nerve regeneration due to the ingrowth of connective tissue following a break in the wall of an arterial sleeve used for peripheral nerve splicing (Weiss, '43).

Failure of functional restoration appears to be due not so much to the complete lack of regenerative cell outgrowths as to the presence of insurmountable obstacles which prevent regenerating nerve fibers from becoming oriented and from growing forward to reach their original terminations.

143. The cerebral cortex of the 3-month infant. J. LeRoy CONEL, Department of Anatomy, Boston University School of Medicine.

The results of this investigation will be published in the third volume of a series of monographs in which the postnatal development of the cerebral cortex is described. Nine criteria are used for evaluating the progress of development. During the time between the first and third months of postnatal life development of the cortex has been fairly uniform in rate in all areas, but growth has been especially active in area FAγ (4) particularly in the region of the hand. In the parietal lobe development has advanced further in area PB than in other areas. In the occipital lobe area OC leads the other areas in development. Area TC is more advanced than other areas in the temporal lobe. Growth has not been as active in areas OC and TC as it has in areas FAγ and PB. Of the horizontal layers of cells, layers V and VI are the most advanced in development.

144. The growth of the brain in the fetal dog. Robert L. CORDER and Homer B. LATIMER. Department of Anatomy, University of Kansas.

Dog fetuses weighing from 6 to 410 gm were used. The method of removal and weighing the brain was the same as that used in similar studies from this laboratory. The brain weight increases from 0.3 to 10 gm, and the cord, from 0.03 to 0.6 gm. The brain weight plotted on body weight forms a straight line curve and the cord weight, a curve very slightly convex superiorly.

The brain was divided into 4 parts and each part was weighed separately. When plotted on body weight, the prosencephalon forms a straight line, and the cerebellum, 2 straight lines, the second rising more rapidly. The mesencephalon also increases as an arithmetical function of the body weight for the first half of the growth period and then remains at a constant level until birth. The medulla forms a curve slightly convex superiorly throughout.

The prosencephalon increases in its percentage of the total brain weight throughout the entire fetal period, but more rapidly at first. The cerebellum likewise increases in its percentage of the brain weight but its curve is concave superiorly, or its increase is more rapid toward the latter part of the fetal period. The mesencephalon and the medulla both decrease in relative weight and both curves are concave superiorly, or the relative changes occur most rapidly in the early part of the fetal period.

145. Changes induced in the anterior pituitary gland of the white rat by prolonged treatment with thiouracil. Alden B. DAWSON, The Biological Laboratories, Harvard University.

The pituitary glands used in this study were obtained through the courtesy of Mr. W. L. Money who has been studying the effects on the thyroid gland of chronic treatment with thiouracil (Money, '46). Adult male rats were maintained on 1.0% thiouracil (in drinking water) for periods up to 16 months. Pituitary glands were taken at 4- to 8-week intervals beginning with the fourth month. At this time ordinary basophiles and thyroidectomy cells were numerous and the acidophiles were but slightly reduced in number. Thereafter, there was ordinarily a rather rapid decrease in unmodified basophiles and a more gradual decrease in thyroidectomy cells. The acidophiles also slowly decreased in number. Maximum reduction of unmodified basophiles and acidophils usually occurred after 10 to 12 months. In late stages of treatment, practically all granular cells disappear and even thyroidectomy cells become rare, approaching the condition found at the end of 40 days, in the glands of rats thyroidectomized at birth (Severinghaus, '42). The deleterious effects of thiouracil are sometimes repaired by the development of a certain type of thyroid adenoma. Single or periodic injections of dibenzanthracene appeared to accelerate the rate of degranulation of the chromophiles and disappearance of thyroidectomy cells. Prolonged treatment with thiouracil may produce effects in the anterior pituitary gland as extensive as those found after total thyroidectomy.

146. The ovary of the pika.[1] Kenneth L. DUKE, Department of Anatomy, Duke University School of Medicine.

The Lagomorpha includes 2 families: Ochotonidae (pikas) and Leporidae (rabbits and hares). Of the genera making up this order the pika is the most primitive. Twenty-six reproductive tracts of *Ochotona princeps uinta* were obtained for study. These animals were trapped during the months of July, August, and September.

The germinal epithelium is cuboidal near the root of the ovary but may become flattened as it approaches the ventral aspect. There are few cellular proliferations from the germinal epithelium into the cortex. The cortical stroma is a compact connective tissue containing many fibroblast nuclei. Some oocytes lie immediately beneath the germinal epithelium, while others are deeper in the cortex. Corpora lutea and large vesicular follicles are present in July. Luteal remnants and numerous growing and small vesicular follicles are common in September. Atresia is widespread during the September period. Call-Exner bodies are seen in the granulosa of the follicles in immature animals. The theca interna, even of vesicular follicles, is thin and fibrous. There is but slight hypertrophy of the theca interna, even in atretic follicles. Interstitial cells are inconspicuous; however, the stroma of immature females contains large epithelial or epithelioid cells. The medullary stroma consists of fibrous connective tissue with few nuclei visible as compared with the cortical stroma.

[1] Aided by a grant from the Duke University Research Council.

147. Some observations on growth of limbs with deficient nerve supply. Donald DUNCAN, Department of Anatomy, University of Texas.

The sciatic nerves of 8 young rats, 10–12 days old, were severely damaged but not completely interrupted by application of tantalum clips. The animals were observed for 140–150 days before sacrifice. During this interval sensibility was present but diminished in all animals and movements of the foot and toes were very feeble or absent. Weights of the leg segments with skin removed were 41–68% less than those on the side with intact nerve supply. The tendons of the undersized muscles were of the same size as those of the opposite side and tibial lengths were not appreciably different except in 1 animal. In this one the tibia of the partially denervated leg was 2 mm (6%) shorter than its mate. The experiments indicate that severe but incomplete denervation of a limb during the post-natal growth period of the species examined affects neither the length of the bones nor the size of the tendons.

148. An histological study of duck limb primordia transplanted to embryonic chick hosts. Herbert L. EASTLICK and N. Karle MOTTET, Department of Zoology, State College of Washington.

Anterior and posterior limb primordia of 26–38 somite duck embryos were transplanted to the flank of 2–3 day chick embryos. This report is based on a study of serial sections of 41 non- or poorly innervated grafts, 5–21 days old.

Premuscle masses are formed in 7 day grafts and subnormal amounts of faintly striated fibrillae appear in 8 day specimens. In 11–18 day grafts the muscle fibers appear to be edematous. Macrophages are numerous between the fibers and sarcolysis and active phagocytosis is taking place. Venous congestion is moderate with some erythrocyte extravasation. Fat organs are developing in the loose connective tissue and most of the fat cells are multivacuolated. In 19 day grafts desquamation of the epidermis and dermal hemorrhage occur. The venous congestion is especially prominent in the subdermal vessels. Fat lobules and loose connective tissue are predominant and little or no muscle remains. In 21 day specimens the fat lobules are composed primarily of univaculate cells. Venous congestion is extensive, edema is prominent, and many erythrocytes are extravasated into the connective tissue between the fat lobules.

149. *Distribution of phosphatase and glycogen in the amphibian embryo.* Herbert ELFTMAN and W. M. COPENHAVER, Department of Anatomy, College of Physicians and Surgeons, Columbia University.

The localization of alkaline phosphatase, acid phosphatase and glycogen has been studied in Amblystoma punctatum embryos ranging from late blastulae to feeding stages (Harrison's stages 8 to 46).

In late blastulae, alkaline phosphatase is present in the yolk granules of both animal and vegetal poles. Acid phosphatase is much more abundant in the vegetal pole, contrasting with glycogen, which is more abundant in the animal pole.

During the development of the heart there is a marked increase in the alkaline phosphatase of the endothelial nuclei. Glycogen is present in the anlage of the heart; at later stages it is more striking in the ventricle than in the atrium. The yolk granules in the heart wall decrease in size and number in the course of development but continue to give a positive phosphatase reaction.

The cells of the retina exhibit a polarity in the distribution of alkaline phosphatase and of glycogen before stage 40. Alkaline phosphatase is associated with developing nerve fibers; in the central nervous system it is differentially distributed. Acid phosphatase is more abundant in the ventral portion of the nerve cord than in the dorsal in the later stages.

The pronephric tubules have large amounts of alkaline phosphatase in their proximal portions, the concentration decreasing distally. Alkaline phosphatase is also associated with the development of the skeletal elements, teeth and the digestive tract.

150. *Factors affecting the estimation of concentration of sperm in ram's semen by the photoelectrometric method.* L. Otis EMIK and George M. SIDWELL, Bureau of Animal Industry, Agricultural Research Administration, U. S. Department of Agriculture. (Introduced by Clair E. Terrill.)

Ejaculates from Rambouillet, Columbia and Targhee rams were used to measure the effects of various factors on estimation of concentration with a colorimeter.

Chlorazene was the diluter of choice, despite its low opacity and change upon prolonged standing, because it alone dissolved clumps which were prevalent in ram's semen. A dilution of 1: 200 gave readings in the optimum range of the colorimeter's scale, and a lower error of estimate.

Scores for estimating visible debris in the samples effected highly significant improvement in estimation of concentration. Residual turbidity, after centrifugation of diluted samples, was not a suitable estimate of debris. Semen with the debris removed gave a regression line through the point of origin.

Two brands of diluting pipettes exhibited significant difference in volume and ability to drain; however the variability of individual pipettes was similar.

The optimum technique (chlorazene at 1: 200 with scoring of debris) gave a standard error of estimate of 37.8 ($\times 10^7$ sperm per ml). The error due to workers' and instruments' variability was negligible. The sampling and dilution errors for the colorimeter and hemacytometer were 21.3 and 21.8, respectively, but the error for the hemacytometer at the working mean was increased to 26.7 due to the Poisson distributional error of the small volume measured. The error due to non-scorable debris and heterogeneity of sample was 34.2.

151. *Development and chromosome number of the offspring of a tetraploid axolotl female mated with a diploid male.* G. FANKHAUSER, Department of Biology, Princeton University, and R. R. HUMPHREY, Department of Anatomy, University of Buffalo.

A 2-year-old tetraploid female axolotl, mated with a diploid male, spawned 120 eggs, of which 98 were fertile. Three eggs died before or during gastrulation; 4 made little progress beyond stage 25. The majority of the remaining embryos showed early abnormalities in the circulatory system, particularly a failure of the blood island to drain into the general circulation. In some of these embryos, however, normal circulation was subsequently established. At stage 40, 49 larvae of fairly normal type were still living. A majority of these developed stasis, hematomata, ascites, etc., or simply refused to start feeding. Of 16 still alive (age 2 months), 2 exhibit extreme edema.

The chromosome number has been determined to date in amputated tailtips of 19 larvae, in which it ranges from 32 to 44, with only 2 larvae showing the expected triploid number, 42. In 5 larvae the number is 41, in 3, 40. The preponderance of unbalanced chromosome complements explains the high percentage of abnormal or poorly viable larvae. It also indicates that irregularities in the pairing of the 4 homologous chromosomes at meiosis are frequent enough in tetraploid oocytes to produce a majority of hypo- and hyper-diploid eggs, and of hypo- and hyper-triploid offspring. The further analysis of this spawning will have considerable theoretical interest since this represents the first breeding test of a tetraploid vertebrate.

152. *The influence of cosmic radiation showers arising from lead plates on the rate of carcinogenesis.* Frank H. J. FIGGE, Department of Anatomy, University of Maryland School of Medicine.

The ultraviolet luminescence spectra of a number of carcinogenic compounds suggested the hypothesis that the action of these substances may be related to their conversion of some form of penetrating radiation such as cosmic radiation to a form of energy capable of inducing intracellular malignant transformations.

To test this hypothesis, 182 inbred mice were injected with 0.5 mg of methylcholanthrene. These mice were divided into 2 groups. The group of 69 control mice in 3 aluminum cages received normal, unmodified sealevel cosmic radiation. The 113 mice in 5 aluminum cages with lead plate covers, were subjected to the normal sealevel cosmic radiation plus the showers of radiation which result from passing cosmic radiation through 1 or 2 lead plates 1 cm thick. The average latent period for the malignant transformations in the controls was 80 days. The average latent period for induction of sarcomas in the 113 experimental mice receiving the intensified cosmic radiation was only 60 days, or three-fourths that of the controls. A repetition of this experiment gave the same results. While these results are significant, experiments to test this hypothesis in a more conclusive manner are in progress.

153. *Pigmentation of adipose tissue as influenced by unsaturated fatty acids in vitamin E deficient rats.* Lloyd J. FILER, Ruth E. RUMERY and Karl E. MASON, Department of Anatomy, University of Rochester School of Medicine and Dentistry.

Accumulations of acid-fast pigment in adipose tissue cells and associated foreign-body reactions previously observed in rats fed vitamin E deficient diets high in cod liver oil (20%), or containing the equivalent of this as the most highly unsaturated fraction of the oil, are not dependent upon the presence of C_{20}–C_{22} fatty acids of fish oils. Comparable feeding of the methyl esters of linseed, soy bean, and corn oils resulted in marked, moderate, and slight cellular reactions in the fat depots, respectively. These experiments indicate that C_{18} fatty acids with 3 double bonds (linolenic acid) are highly effective in producing these histological changes, while C_{18} fatty acids with 2 double bonds (linoleic acid) exert much lesser, but similar, effects if fed at sufficiently high levels for long enough periods of time. Compared to lard, the unsaturated fats mentioned markedly accelerate dystrophic changes in skeletal muscles and kidney nephrosis.

In rats fed E-deficient diets containing 10% methyl esters of linseed oil and given graded, oral doses of purified natural tocopherols, from 30 to 75 days of age, the daily intake of alpha tocopherol (0.2 mg) and of gamma tocopherol (2.0 mg) just sufficient to protect against the alterations in adipose tissue cells also proved to be critical levels for preventing muscle and testis injury. Other antioxidants (ascorbic acids, gallic acid, propyl gallate, nordihydroguaiaretic acid) proved ineffective.

154. *The motor and sensory fibers in the rootlets of the seventh cranial nerve of the cat.* James O. FOLEY, Department of Anatomy, Medical College of Alabama.

The roots of the right seventh cranial nerve were sectioned intracranially in 10 cats and, after allowing 14 days for degeneration of the motor axons, counts of the fibers in the right nerve proximal to the geniculate ganglion were made and compared with similar analyses of the left seventh nerve of the same animals.

The enumerations showed that there were from 6902 to 10276 special visceral efferent axons, 890 to 2551 general visceral motor axons and 1363 to 3175 sensory

nerve fibers in the proximal part of the seventh cranial nerve of the 10 cats. Expressed in terms of percentages, the special visceral efferent axons ranged from 57.16 to 79.13, the general visceral efferent fibers from 8.18 to 20.93, and the sensory axons from 12.51 to 25.81.

When the separate values for the different functional components of all nerves were averaged, it was found that the special visceral efferent component was represented by 8506 axons (68.86%), the general visceral efferent component by 1854 fibers (15.01%), and the sensory component by 1994 axons (16.13%).

155. Ceroid pigment in the testis of mice treated with estrogens. Marthella FRANTZ, Department of Anatomy, University of Minnesota. (Introduced by Arthur Kirschbaum.)

Brown pigment has been reported to appear in the testes of mice treated with estrogenic hormone (Hooker and Pfeiffer, '42) and of vitamin E deficient rats (Mason and Emmel, '44). Pigment of this type has also been reported to occur spontaneously in the testes of many species of mammals, especially in animals of relatively advanced age.

Lillie, Daft and Sebrell ('41) observed a brown pigment in hepatic cirrhosis of rats induced by dietary means, which they designated as "ceroid." In hematoxylin-eosin sections the testicular pigment of mice treated with estrogens appeared to be similar. In order to establish the ceroid character of this testicular pigment, sections of testis of estrogen-treated Bagg albino mice were stained with the methods of Endicott and Lillie ('44). The pigment's ceroid nature is indicated by its acid-fastness, sudanophilia, stainability in dilute aqueous solutions of basic aniline dyes (basic fuchsin, methyl and crystal violet, methyl green, safranin O, methylene and toluidin blue), its lack of stainability with similar solutions of acid aniline dyes (orange G, Congo red, trypan blue, acid fuchsin, eosin Y and B, and Phloxine B), and its insolubility in common fat solvents.

156. The effect of amputation of the dorsal fin upon the development of Sd melanomas in hybrid fishes. E. D. GOLDSMITH, Myron GORDON and Ross F. NIGRELLI, Department of Anatomy, New York University College of Dentistry and New York Aquarium, New York Zoological Society.

Hybrid fishes (Platypoecilus — Xiphophorus) carrying the Sd gene develop melanomas of the dorsal fin.

The dorsal fins of 8 6-week-old, 6 3-week-old, 8 2-week-old fish were amputated at the base. At these ages the fins had few or no macromelanophores. The fins regenerated completely within 2 weeks and were removed again at that time and at 2-week intervals for a total of 10 amputations. In the 2-week interval few or no macromelanophores developed in the regenerated fins, although a number did develop on other parts of the body. Within a month following the last amputation, most of the dorsal fins developed some pigmentation. Continuous observations for 5 additional months disclosed additional pigmentation in these fins. However, it was far less than in the unoperated individuals. In fact, the dorsal fins of unoperated control fish, 5 months of age, had more pigment than the regenerated fins of 11-month-old animals.

The explanation for the difference might lie in the fact that the tissues comprising the regenerated fins are chronologically younger than that in the controls. Further work is in progress.

157. The excretion of fluorescein by the liver under normal and abnormal conditions, observed in vivo with the fluorescence microscope. Allan L. GRAFFLIN,[1] Department of Anatomy, Harvard Medical School, and Department of Medicine, College of Physicians and Surgeons, Columbia University.

All observations were made upon male specimens of *Rana pipiens*, receiving fluorescein (uranine) in standard dosage of 30 mgm per kilogram. After injection the dye appears promptly in the plasma, then in the hepatic cells and the bile capillaries. The peak of excretion (all bile capillaries sharply delineated, high intensity; all hepatic cells diffusely stained, low intensity) is reached in approximately 1 hour at ca. 15°C. The sequence of events from the peak of excretion to complete elimination is essentially a progressive diminution in fluorescent intensity of the dye to non-detectable levels, achieved first in the hepatic cells, then (much later) in the bile capillaries. Elimination is usually complete in 24 hours or less. The present observations upon the normal mechanism of fluorescein excretion have failed to confirm the findings of Hirt, Ansorge and Markstahler (Z. f. Anat. u. Entw., *109:* 1, 1939) in the later stages of excretion (extensive sprouting of fluorescein-containing vacuoles from bile capillaries, widespread intracellular vacuole formation, discontinuity of bile capillaries, "lakes" of fluorescein). However, the whole range of these findings has been observed in animals subjected to highly abnormal conditions, with anoxia probably the most important single factor. A refractory period of at least several hours' duration, as insisted upon by Hirt et al., does not occur, with respect to fluorescein excretion, in the livers of frogs maintained under normal conditions.

[1] Senior Fellow in Cancer Research, American Cancer Society, recommended by the Committee on Growth, National Research Council.

158. Histogenesis of Mauthner's neurone in Amblystoma. Walter F. GREENE, Syracuse University and Osborn Laboratory, Yale University.

The differentiation of this intercalated neurone can be correlated with the morphologic stages of Harrison and the motility phases described by Coghill. The early location of this neuroblast is constant and definite among the marginal tracts of the medulla. Axone growth and decussation; Nissl bodies; pigment migration; and dendritic field become apparent in synchrony with the total reaction pattern. The dendritic field becomes elaborate as the sensory stimulation (acousticolateralis inflow) is perfected. Axonal growth extends caudad along the brain stem in advance of ganglionic and receptor maturity of the labyrinth and lateral line organs. Experimental extirpation of its synaptic primary sensory neurones can fractionally deplete the lateral dendritic field of Mauthner's cell. In such cases the relations to the motor pattern of the medulla remain intact. Several cases are recorded in which this intercalated neurone was absent from the operated side of the medulla. Decussation of the remaining axone was normal in most of these cases.

159. The acid phosphatase reaction in degenerating axons of certain sensory tracts. Walter L. HARD [1] and Bernard FERRARA, Department of Anatomy, Medical College of the State of South Carolina.

The presence of acid P-ase in the axons of both sensory and interstitial neurons of the CNA is reported. The sensitivity to injury of pyramidal tracts fibers in the cat and monkey has been identified by the disappearance of P-ase from the injured axon (Hard and Lassek, '46; Lassek and Hard, '46). The present study extends these observations to fibers of the trigeminal, spinocerebellar, and gracili fasciculi.

Axonal injury was produced in a total of 33 cats by appropriately placed lesions in the medulla (15), low thoracic cord (10), and dorsal roots (8). Phosphatase disappeared from fibers of the descending trigeminal tract within a 32-hour postoperative time interval. In hemisected animals, as well as dorsal root sections, maximal loss of P-ase occurred in the posterior funiculus by a 3-day postoperative time. However in these hemisections the spinocerebellar tracts showed little or no (4 animals) degeneration at 3 days, extensive degeneration at 4, and maximal loss at 5 days postoperative times. In hemisections the degeneration of cortico-spinal fibers during a 3-day postoperative interval confirms earlier work. These observations reflect a distinct variation in susceptibility to injury of diverse neurons. Studies are in progress to determine if variations in enzyme concentrations of the diverse neurons is alone sufficient to explain this type of response.

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160. Changes in mitochondrial content of motor nerve cell bodies following axone section. J. Francis HARTMANN, Department of Anatomy, University of Minnesota.

In this experiment, the right sciatic nerve was cut in a series of 19 rats, after which animals were killed at daily intervals from the second through the tenth postoperative day, perfused with Regaud's fluid, and the appropriate segments of the spinal cord removed for study. Alternate 3 μ cross sections of the cord were stained for Nissl substance with carbon-thionin and for mitochondria with the basic fuchsin technique of Fain and Wolfe. Beginning with the seventh postoperative day, a definite increase in fuchsinophilic granulation is evident in the chromatolyzed cells in the ventral horn of the operated side as contrasted with the control side. Counts of mitochondria in affected and normal cells show an average calculated number of 170.4 million per mm^3 on the operated side as opposed to 90.2 million on the control side on the tenth postoperative day. Direct comparison of Nissl-stained sections of individual cells with adjacent sections through the same cells stained for mitochondria reveals that the increase in fuchsinophilic granulation follows approximately the same time course as does chromatolysis. The increased red granulation in the affected cells is thought to be due to increased numbers of mitochondria rather than to degraded Nissl material staining with basic fuchsin because the granulation is as intense in the axone hillock as elsewhere in such cells.

161. The plasmal reaction. E. Russell HAYES,[1] Department of Anatomy, The Ohio State University. (Introduced by R. A. Knouff.)

The plasmal reaction (PR) of Feulgen and Voit ('24), if applied rigorously, can be used for the strict demonstration of acetal-lipids. The bases for a rigorous PR are: (1) obtaining a negative control section from the same block of tissue, which allows precise definition of the unmasking mechanism; (2) restricting treatment of the fresh tissue sections to 2–5 minutes in $HgCl_2$; this, by decreasing the time element, minimizes the unmasking effects of acid and of oxidation and thus takes full advantage of the high specificity of $HgCl_2$ for the acetal bond. Formol fixation rapidly diminishes and finally destroys the "true" PR and progressively develops a secondary reaction with fuchsin-sulfurous acid that is independent of $HgCl_2$. In normal rat adrenal and liver these 2 carbonyl-lipid reactions are so markedly different in distribution that it seems clear that formol fixation (oxidation?) is not equivalent to $HgCl_2$-unmasking and probably demonstrates carbonyl-lipids other than acetals. Observations indicate that this "true" PR is rather stable in the rat adrenal under stresses that markedly alter the Sudan and Schultz reactions.

[1] Now at the University of Buffalo.

162. Some observations on the human nervus vertebralis. Edward A. HOLYOKE and Alister I. FINLAYSON, Departments of Anatomy and Surgery, University of Nebraska College of Medicine.

Although the nervus vertebralis is frequently a well-defined feature of the sympathetic system in the cervical region it has been entirely ignored in most standard works on anatomy and has received scant attention in the literature. There is little agreement as to its frequency, connections, or significance.

The vertebral nerve has been observed in a series of 12 out of 20 adult cadavers during the present routine course in gross dissection. It was found on one side, usually the right, in 8 cases and on both sides in 4 (making 16 nerves observed). It is a nerve of variable size passing superiorly from the inferior or occasionally (6 instances) the middle cervical ganglia to perforate the prevertebral fascia and longus capitis and cervicis muscles. In accord with Broek (except 1 case) it crosses ventral to the vertebral artery from lateral to medial to communicate in most cases with the fifth, sixth, and seventh, or sixth and seventh cervical nerves. Two cases communicated with the fifth and sixth and one with the fourth and fifth nerves. In one instance there were 2 separate nerves communicating respectively with the fourth and sixth cervicals.

We do not yet have evidence as to the functional significance of this nerve. It does not seem to connect extensively with the sympathetic plexus of the vertebral artery.

163. Temperature influence during embryonic development in rats. C. Y. HSU, Department of Anatomy, Washington University School of Medicine, St. Louis, Mo. (Introduced by John H. Van Dyke.)

Pregnant rats (Wistar) were incubated 0.5–10.5 days after insemination at 39°C. for 1, 2 or 4 hours. Rectal temperature, following treatment, averaged 5–6°F. above normal.

Six females treated for 1 hour 0.5–1.5 days after insemination and 6 animals subjected for 2 hours during the same gestation periods failed to produce young, with the exception of 2 cases. Delivery in one was normal; in the other, 2 offspring were dead. Parturition in animals treated similarly during advanced stages of pregnancy was normal.

However, with the exception of the following individuals, a third group of females (12) subjected for 4 hours 0.5–10.5 days after insemination gave positive results. Three animals (4.5, 5.5 and 6.5 days pregnant) delivered no offspring. Another 6.5 day specimen produced a litter of 10 (2 twins, 2 within fetal membranes, dead; the remaining apparently normal). Uteri of a third 6.5 day pregnancy were examined immediately before delivery (5 were completely resorbed, 3 partially resorbed and 3 apparently normal).

Examination of corpora lutea during pregnancy in this preliminary study suggests that, despite certain individual variations, increase in body temperature for a relatively short period may terminate early pregnancies, that the period immediately before, during and just after implantation is more susceptible to severe heat treatment, and that later stages of gestation are most resistant to temperature changes.

164. The suprahyoid hypoglossal ansa (of Hyrtl): a nerve crossing the midline of the body. Robert M. JAMESON and William O. REINHARDT, Department of Anatomy, University of California Medical School.

Instances of anastomosis across the midline of the body of peripheral or cranial nerves in man are of sufficient rarity to warrant attention. The present example is one in which the 2 hypoglossal nerves were continuous across the midline in relationship to the antero-superior aspect of the hyoid bone. This loop or ansa (found in a 49-year-old male cadaver) was approximately one-third the diameter of either of the main roots of the hypoglossal nerves. The loop maintained a uniform diameter across the midline, gave off a few fine twigs to the geniohyoid muscles on either side of the neck, and traversed in its course the deep cervical fascia in immediate relationship to the antero-superior aspect of the hyoid bone. Attention has been directed to this variation by Hyrtl, who named it the suprahyoid hypoglossal ansa, distinguishing it, thereby, from the infrahyoid cervical ansa of the hypoglossal nerve. The ansa was first described by Bach in 1835, has been seen by Arnold (1851), and by Hyrtl (1865). This suprahyoid loop or ansa of the hypoglossal nerve has not been studied in any previously cited case as to the origin of its contained fibers. It is possible that the suprahyoid ansa may be of cervical rather than hypoglossal origin, and might, therefore, be

more properly designated as a suprahyoid cervical ansa. Such extra-cranial anastomoses across the midline of the body have been noted more constantly in other forms, i.e., the chiasmatic anastomosis of the internal laryngeal nerves in turtles, and the hypoglossal anastomosis in the suprahyoid region of the alligator and the ostrich. The incidence of this variation has been quoted at 10% on the basis of Bach's original findings (1835) of 3 cases in 32 subjects examined. In view of the paucity of further reports of instances of this loop formation, a frequency of 10% is certainly too high.

165. The Golgi element in primitive erythroblasts of the 11-day rat embryo. Oliver P. JONES, Department of Anatomy, University of Buffalo.

Preliminary observations indicate that structures similar to the Golgi element described by J. R. Baker ('44) are also preserved in dry smears of embryonic blood. These may be stained supravitally by immersing the yolk sac in an isotonic salt solution of dahlia violet. Portions of these structures have a strong affinity for Sudan black after dry smears have been fixed in formol-calcium. With phase microscopy some of these forms are true discs or rings which may appear rod-like, elliptical or circular depending on their orientation. The centers, which are sudanophobic, correspond to neutral red vacuoles. Fetal livers from 15-day embryos are also suitable for similar studies on the definitive generation of erythroblasts.

166. On the nature of Auer rods.[1] Raymond S. KIBLER, Department of Medicine, University of Buffalo and the Buffalo General Hospital. (Introduced by R. R. Humphrey.)

Early myeloid cells of 5 cases of acute myeloblastic leukemia have been studied by means of a combined Sudan black-Romanowsky technique. Auer rods exhibited an intense sudanophilia; the largest rods possessed a sudanophobic core. It was observed that Auer rods were present in each case and that they occurred in a much greater percentage of the promyelocytes than could be demonstrated by a standard Romanowsky stain (May-Grünwald-Giemsa). In many instances within the same promyelocyte there were demonstrated all stages of Auer rod development from an intensely sudanophilic granule to the largest rod. Phase microscopy appeared to confirm the observation that the largest Auer rods possessed a well-defined cortex in which the lipoid was concentrated, and that the presence of a sudanophilic cortex is not an optifact. The Kurloff bodies of the guinea pig and lymphoid azure granulation to which the Auer rods have been likened were shown to be constantly sudanophobic. Differential extractions indicated that the contained lipoid was a phospholipid. It is suggested that the rod-like structures reported in a variety of disorders of the blood and/or bone marrow resemble Auer rods only in their morphology and differ from the genuine Auer rods of myeloid leukemias in chemical composition.

[1] Aided in part by a grant from the Ciba Pharmaceutical Company.

167. Morphological evidence suggesting that human extrinsic eye muscles are phylogenetically relatively primitive. Hadley KIRKMAN, Department of Anatomy, Stanford University.

In a total of 30 cadavers examined 73% showed macroscopically visible nodular swellings near the middle of the bellies of some or all of the extrinsic eye muscles. No similar swellings were observed in any other skeletal muscles examined.

From a second series of 35 cadavers a total of 37 extrinsic eye muscles and a total of 70 extra-ocular skeletal muscles, including lumbricales of index fingers, posterior venters of digastrics, tensor tympanis, and one levator palpebrae superioris, were examined microscopically. In 36 of the eye muscles the swellings were due to accumulations of localized contraction nodes in single muscle fibers. Similar nodes were observed in but one of the extra-ocular muscles (one of 12 tensor tympani muscles).

The nodes were independent of the state of rigor since the specimens were embalmed from 6 to 168 hours post mortem.

Localized contraction nodes are characteristic of smooth muscle, are common in skeletal muscle of insects and lower vertebrates, and occur in denervated mammalian skeletal muscle. In striated muscle they are generally interpreted as a relapse to a smooth muscle type of contraction.

It is concluded that the morphological observations reported here furnish data, in addition to those already available in the literature, in support of the hypothesis that mammalian extrinsic eye muscles are phylogenetically more primitive than other mammalian skeletal muscles.

168. Topography of fibers in the medullary center of the human cerebellum. Wendell J. S. KRIEG, Institute of Neurology, Northwestern University Medical School.

Fiber-stained sections are inadequate to show the complete course of fibers of the medullary center of the human cerebellum and Marchi experiments are impracticable. However, fiber dissection is quite feasible on the decorticated cerebellum after certain treatments.

Brachium pontis is shown to distribute to lateral lobe generally but in diminished numbers to its inferior part. Deep rostral fibers make a sharp bend forward and medially to supply anterior lobe, fanning out medially and laterally. Fibers to lateral part of superior semilunar lobule form superficial stratum of pons, converge in region of trigeminal root. Those which reach medial part of lobule run medially anterior to dentate nucleus, then turn backward within their folia. Fibers to inferolateral part of lateral lobe are superficial and caudal in pons, while those to inferomedial part are deep but not necessarily caudal.

Restiform body can be traced as a unit throughout medullary center. As soon as it has cleared brachium pontis deeply, fibers turn backward to pass to superior semilunar lobule. This tract continues medially just in front of hilus of dentate, sending fibers backward to middle lobe and forward to anterior lobe. Component to middle vermis curves backward around medial edge of dentate.

(For remainder of connections see abstract of demonstration.)

169. The lateral infracostal artery. Benjamin N. KROPP, Department of Anatomy, Queen's University, Kingston, Ontario.

In a series of 82 dissection room subjects, the frequency of occurrence and relations of the lateral infracostal artery were noted. Typically this artery is a branch of the internal mammary and descends obliquely and laterally close to the mid-axillary line deep to the parietal pleura. It arises near the origin of the internal mammary, rarely from the subclavian, and may traverse 6 intercostal spaces. A vein accompanies the artery, and both communicate with the corresponding intercostal vessels of the spaces they cross.

The artery has been mentioned under a variety of names, A. thoracica interna, Hodges, 1858; A. mammaria interna lateralis, Henle, 1868; and others. Adachi, 1928, found it on the right side alone in 8.1% and on the left side alone in 14% of all cases. In the present series, the origin and course of the vessel conformed in general to earlier descriptions. The distribution, however, was found to be variable and the incidence markedly higher than previously reported. In 42.6% of all cases, the artery was present on one or both sides; in 14.6% on the right side alone, in 18.2% on the left side alone, and in 9.7% on both sides. When present it reached the fifth intercostal space in 30% of cases. The occurrence of the artery has no apparent relation to the sex or age of the subject.

170. Components of supporting tissue and ganglion cell capsules in autonomic ganglia.[1] Albert KUNTZ and Norman M. SULKIN, Department of Anatomy, St. Louis University School of Medicine.

Sections of autonomic ganglia of the cat and of man have been prepared by various methods including Masson's trichromic technic, Quade's ferric gallate stain, Cajal's gold chloride sublimate method for astrocytes and the methods of Del Rio Hortega, Penfield and Grino for oligodendroglia and microglia. The data obtained in a study of these preparations support the following conclusions: The supporting tissue within the ganglia normally includes but few collagenous elements. The ganglion cell capsule typically consists of a single layer of flat cells with few cytoplasmic processes. Most of the cellular components of the interstitial tissue are similar in cytologic structure, size and staining reactions to the capsule cells. On the basis of these characteristics which they possess in common with oligodendroglia the capsule cells and the related interstitial cells appear to be homologous with the oligodendroglia in the central nervous system.

[1] This work has been supported by a grant from the Life Insurance Medical Research Fund.

171. Acid phosphatase in peripheral degenerating and regenerating neurons. A. M. LASSEK and E. D. BUEKER, Department of Anatomy, Medical College of the State of South Carolina.

The reaction of acid phosphatase in motor cells and sciatic nerve axons has been studied in 53 animals (carnivora, aves and amphibia) following either high crushing or sectioning. The following was found in a series of cats: The enzyme disappears from the distal portion of the severed axons rather com-

pletely between the third and fourth postoperative days whereas it remains intact in the central stump during the entire degenerative and regenerative cycles. Acid phosphatase accompanies the growing nerve and is quantitatively reduced during the maturation of the growing axons. Its speed of advance peripherally is much faster in the crushed than sectioned nerves. We have detected an increase of acid phosphatase in affected anterior horn cells as early as the first and as late as the eighty-fourth days (the latest sacrifice time to date). Maximal results are obtained in the cells between the twelfth and eighteenth days. Injections of commercial formalin or gentian violet into the central stump at the time of section has not reduced the early heightened acid phosphatase activity in the involved motor cells in preliminary experiments. The reaction is also present in chickens and frogs under similar experimental conditions. It is concluded that the enzyme, acid phosphatase, is present in all stages of nerve regeneration in the animals studied.

172. Electrophoretic study of liver cytoplasmic proteins. Arnold LAZAROW and Jack BERMAN, Department of Anatomy, Western Reserve University.

Guinea pig livers were freed of blood by perfusion through the inferior vena cava. The liver cells were fragmented by squeezing the liver through bolting silk, and the resulting liver brei was suspended in 1.5 volumes of 0.85% saline. The nuclei, mitochondria, and submicroscopic cytoplasmic components were removed by differential centrifugation, and final clarification was accomplished at 18,000 × g. for 60 minutes, using the multispeed attachment for the International centrifuge. The resulting supernatant, a clear amber liquid, showing practically no opalescence, was dialyzed against veronal buffer pH 8.6 and ionic strength of 0.1. The electrophoretic pattern was measured in the Tiselius apparatus. Control studies were carried out on the serum obtained from the same guinea pig.

A number of distinct components were observed in the electrophoretic pattern of the liver proteins. On comparing the serum and the liver patterns, it was noted that the fastest liver component migrated at about 65% of the rate of serum albumen. Purified guinea pig serum albumen was added to the liver protein solution before electrophoresis (20% albumen to 80% liver proteins). The pattern of the mixture revealed that the added albumen migrated at its normal rate, and the fastest liver component migrated at 64% the rate of the added albumen. Studies are being carried out to determine whether the fastest moving liver component is a complex of albumen.

173. Gonadotrophic hormone content of rat pituitaries following thiouracil and testosterone propionate. James H. LEATHEM, Department of Zoology and Bureau of Biological Research, Rutgers University.

Eight adult male rats were fed 0.5% thiouracil in the stock diet for 20 days and served as the control for food intake. Three additional groups of 8 rats each were pair fed and included rats given thiouracil and 0.5 mg testosterone propionate (perandren, Ciba) daily; normal rats and normal rats injected with the androgen. Pituitaries were macerated in a centrifuge tube to which was added distilled water and a drop of 2% NaOH. Littermate recipients were 22-day-

old female rats which were injected twice daily for 4 days and killed 24 hours later. Ovarian weight increase was the criterion of response; each rat received but 1 pituitary. Ovarian weight in recipient rats was increased from 13 mg to 70 mg by 1 pituitary from thiouracil-fed rats and to 65 mg by a pair fed control pituitary indicating the absence of an effect. Rats fed thiouracil and injected with androgen increased ovarian weight to only 28 mg with 1 pituitary but a similar reduction in hormone content occurred with pair fed androgen injected normals whose pituitaries increased ovarian weight to 31 mg. Since the pituitary weights were essentially the same, thiouracil did not alter the gonadotrophic hormone content or the effect of the androgen when compared with pair fed controls.

174. The epicytes of mammalian lungs. Charles C. MACKLIN, The University of Western Ontario Faculty of Medicine, London, Canada.

These scattered entodermal epithelial cells, also known by other names, as septal cells, were found universally distributed in the alveolar walls of healthy lungs of 10 mammalian species including man, in such numbers as to suggest that they would make a substantial mass if merged. The form is much altered by fixation methods. Most favorably displayed in material fixed by vascular perfusion, the profile resembles a carafe, with the larger end containing the nucleus, around which is the most conspicuous feature, a zone of clear bodies or vacuoloids. When epicytes show phagocytosis the ingested dust particles or dye molecules are found not in the vacuoloids but in the cytoplasm between them. Epicytes which extend through the partitional alveolar walls have 2 free surfaces, a larger and a smaller; and both of these in silver-washed material may be surrounded by silvered rings and sprinkled with silvered granules. They lie between larger areas often regarded as containing epithelial squames, and occupy sockets bordered by capillaries which frequently indent their sides. They have been found apparently being released from their sockets to become free dust or foam cells. Epicytes resemble the epithelial cells of the respiratory bronchioles, where cell diversification is apparently proceeding. After fixation by bronchial injection they are flattened, and after immersion fixation they are usually so buried in the surrounding tissue as to be overlooked.

175. Failure of the theory of partial sex-linkage to explain known facts of inheritance of xeroderma pigmentosum. Madge T. MACKLIN, Department of Zoology-Entomology, The Ohio State University.

If a recessive gene is partially sex-linked, at least 1 crossover must occur in 75% of families producing affected sons when parents are related, and 3 crossovers must occur in 25% of families. The crossovers must occur at specific places somewhere between the ancestor with the affected gene and the affected son. In similar matings, producing affected females, 62.5% require 2 crossovers, 6.25% 4 crossovers, and 31.25% require none. Haldane sets the preferred crossover value for xeroderma at 14, mentioning 35 as an upper estimate. With 14 as the value, 25% of consanguineous matings should produce affected males, 75% affected females. Of 53 recorded consaguineous matings, 29 produced only affected males, 24 only affected females. At a value of 14, χ^2 is 23; at 35, χ^2 is 3.46. The

first excludes the hypothesis of sex-linkage, the second not quite. Tests for linkage with sex in families in which the affected paternal grandparent can be identified on this hypothesis, give χ^2 of 0.5. The recorded data do not support the hypothesis of partial sex-linkage for the gene causing xeroderma pigmentosum. The family regarded by Haldane who did not know the relationship of the parents as most strongly supporting partial sex-linkage requires 7 crossovers to produce the 7 affected males.

176. *An adrenergic link in the ovulatory mechanism of the rabbit.* J. E. MARKEE, Charles H. SAWYER and W. Henry HOLLINSHEAD, Department of Anatomy, Duke University School of Medicine.

The results of our previous experiments with electrical stimulation to determine the nature of the control of the nervous system over the hypophysis have indicated that the hypothalamico-pituitary pathway probably contains a non-nervous link. In the present work the known neurohumoral agents, acetylcholine and adrenaline, have been administered to mature female rabbits both intravascularly and by direct instillation into the anterior hypophysis. Intravenous injection of acetylcholine did not induce ovulation in any of 7 animals. Ovulation also failed to occur in 22 of 23 animals in which acetylcholine was instilled into the hypothesis; it is therefore unlikely that acetylcholine is concerned with the release of luteinizing hormone in the rabbit.

Intravascular administration of adrenaline in 20 animals was not followed by ovulation. However, direct instillation of adrenaline into the hypophysis produced ovulation in 12 of 51 animals in all dilutions used, and 5 of 10 with 1/1000 dilution. There was no correlation between the amount of hypophyseal damage observed and the occurrence of ovulation. The effect of adrenaline could not be attributed to lowered tissue oxidation, for neither systemic anoxemia nor injection of potassium cyanide into the hypophysis produced ovulation.

The present experiments are interpreted to indicate that the humoral link in the hypothalamico-pituitary path involved in ovulation is of an adrenergic nature.

177. *The scalenus minimus muscle: its occurrence and morphology in Brazilian Whites and Negroes.* J. P. P. MELLO,[1] Medical School, University of Brazil, Rio de Janeiro. (Introduced by N. L. Hoerr.)

Forty-six individuals (26 males and 20 females), totalling 92 sides of Brazilian Whites (21), Mulattoes (16) and Negroes (9), were dissected. The scalenus minimus was found in 63% of the individuals and in 45.6% of the sides considered. The total number of muscles was 38, out of which 69% were unilateral (70% on the right, 30% on the left), and 31% bilaterally.

There are strong indications that the occurrence of the scalenus minimus is influenced by racial factors, the Whites appearing to have the highest ratio (70%), the Negroes the lowest (40%), with the Mulattoes in an intermediate position (55%).

No indication of sex variation was seen.

All of the 38 muscles studied originated on the seventh cervical vertebra. In 70.2% of the cases, this was the only origin. In 25.43%, an additional origin

on the sixth cervical vertebra was noted, and in only 4.25% another slip came from the neck of the first rib.

The insertion was in all cases the inner margin of the first rib. Sometimes the tendon of insertion was observed to spread out upon the pleural dome, but careful dissection always succeeded in separating this tendinous expansion from the endothoracic fascia. In cases of a well developed muscle, the first rib showed a small spine on the inner border, corresponding to the muscle insertion.

Concerning relations, the muscle lay always between the subclavian artery and the lowest trunk of the brachial plexus.

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[1] Commonwealth Fellow in Anatomy, Department of Anatomy, Western Reserve University, School of Medicine.

178. *Effects of thiouracil, estradiol and testosterone propionate on the time of appearance of the ossification centers of the rat.* Charles R. NOBACK, Department of Anatomy, James C. BARNETT, Department of Radiology, Long Island College of Medicine and Herbert S. KUPPERMAN, Department of Experimental Medicine, University of Georgia, School of Medicine.

A delay of from 1 to 5 days in the time of the appearance of the epiphyseal ossification centers of the extremities of the rat results after the daily injection of thiouracil (deracil). Rats received daily subcutaneous injections of 1–1.25 mg of thiourea for 10 days after birth and of 2.5 mg of thiourea thereafter. Roentgenographic studies were made of the ossification centers which appear normally 8 to 10 days after birth. The 5 litters examined were divided into 10 control and 16 experimental animals. Effects of thiourea were indicated by the poor growth of the animals and by cellular hyperplasia of the thyroid glands noted after the animals were sacrificed.

The daily subcutaneous injection for the first 9–10 days of life of 12.5 μ of estradiol in sesame oil or of 125 μ of testosterone propionate in sesame oil into either male or female rats does not influence the time of appearance of the same centers studied above. A total of 30 litters consisting of 93 control and 87 experimental animals were studied. The animals were sacrificed at 9–10 days of age, cleared and stained with alizarin red for the examination of the skeleton. Wet weights of the uteri and seminal vesicles were used to determine the activities of the hormones.

Littermate animals of the same sex served as controls in all experiments.

179. *A third pressoreceptor area of the body: afferent nerve endings in the coeliac and superior mesenteric arteries.* José F. NONIDEZ, Department of Anatomy, Cornell University Medical College.

The experiments of Heymans, Bouckaert, Farber and Hsu[1] in the dog point to the existence of pressoreceptors for the splanchnic circulation the function of which can be demonstrated after afferent denervation of the carotid sinus and the arch of the aorta. The work here reported shows that these pressoreceptors occur at the bases of the coeliac and superior mesenteric arteries, respectively; they are terminations of medium-sized nerve fibers branching in the externa

as well as in the outer half of the media of these arteries. The branches of arborization are longer than in the corresponding terminations of the carotid sinus and the aorta, and lack the pronounced reticulated swellings found in those areas; since they are so extensive they are seldom included within 1 section. Arborizations of this type do not occur in the descending aorta, nor are they present in the renal, inferior mesenteric and spermatic arteries.

The origin of the splanchnic pressoreceptor fibers has not been determined. They probably reach their destination by way of the splanchnic nerves via the coeliac plexus because, as shown by Heymans and coworkers, the splanchnic vascular reflexes are abolished after acute destruction of the spinal cord. The silver nitrate method of Cajal (chloral hydrate fixation) was used.

[1] Spinal vasomotor reflexes associated with variations in blood pressure. Am. J. Physiol., *117:* 619–626, 1936.

180. Malformations produced by sodium thiocyanate in the early development of the chick embryo. Wiktor W. NOWINSKI[1] and José PANDRA. From the Institute of General Anatomy and Embryology, Faculty of Medicine, University of Buenos Aires. (Introduced by Charles M. Pomerat.)

Sodium thiocyanate in concentrations ranging from 0.4–24.2 mg was injected into the vitellin sack of unincubated hen-eggs and then incubated for 40–48 hours. In this way a series of malformations was obtained of which the following types occurred regularly: (1) Omphalocephaly, accompanied in the great majority of cases by a non-individuation of the cell material in the region of the forebrain and midbrain; (2) The neural plate in the region of the metencephalon did not form the neural tube. This malformation is accompanied by a stretching of the notochord in the vertical direction; (3) Due, in all probability, to the change in growth rate of the neural folds, a superposition of the 2 neural folds was formed. In this case, the fold which grew inside the neural canal, continues to grow in 3 directions: towards the bottom of the neural tube and along its lumen. The slowly growing fold, once in contact with that of the other side, stops growing. From this fact the conclusion is drawn that the factor which regulates the closure of the neural tube is simply a mechanical one: when the 2 borders touch each other, their further growth is inhibited.

The above mentioned malformations occurred either alone, or in combinations, the third one being the most frequent.

[1] Now in the Department of Anatomy, University of Texas Medical Branch, Galveston, Texas.

181. The physiology of the tubal sac of the oviduct of the rat. D. Louise ODOR and Richard J. BLANDAU, Department of Anatomy, University of Rochester, School of Medicine and Dentistry.

The dilatation and muscular activity of the tubal sac of the oviduct of the rat during the period of behavioral estrus has been investigated. It was found that dilatation of the sac begins at the onset of heat and continues at a gradual rate until maximal dilatation was attained 6 to 8 hours later. A definite constriction

forms at the distal end of the tubal sac as the dilatation proceeds. Muscular activity of this portion of the oviduct, at the onset of heat, consists of pronounced pendulum-like contractions which occur at the rate of 10 to 15 per minute. Four to 6 hours later these pendulum-like contractions subside and are replaced by strong peristaltic waves which sweep along the tubal sac and end in the region of the distal constriction. Particulate matter or freshly ovulated ova inserted into the periovarial space are propelled into the proximal end of the tubal sac by the ciliary activity of the fimbria. Their further progression into the distal part of the sac is accomplished by strong peristaltic waves which carry the material in the lumen forward at a rapid rate. Once the particulate matter has reached the distal end of the sac it remains more or less stationary, the distal constriction preventing any further forward progression of the material in the lumen.

182. Cell counts in the anterior pituitary of the cat. Clinton M. OSBORN, Department of Anatomy,[1] The Ohio State University.

Hypophyses of 35 cats representing controls, inanition, and acute and chronic adrenal insufficiencies were fixed by the method of Dawson and Friedgood, '37. Serial sections cut frontally or sagittally at 5 micra were stained with Mallory-Azan or Masson. Outline drawings of every twentieth section were made by projection on millimeter graph paper and squares labeled to coincide with ocular micrometer squares. Using O.I. objective with 10 × ocular, total counts of cells displaying nucleoli and within the limits of the micrometer field were recorded in appropriate squares of the graph paper. By such a "checkerboard" recording, a quantitative picture showing cell type distribution over the entire section or any part of a section may be obtained. Data concerning cell type distribution in 3 dimensions are available by combining graphic records of appropriate consecutively counted sections.

Counts averaging 30,000 cells per gland indicate for acidophils, basophils and chromophobes respectively 42%, 16.5%, 41.5% (normal female sacrificed 2/27/40); 42.2%, 19.8%, 38% (normal male sacrificed 3/18/40); and 36.3%, 10.8%, 52.9% (adrenalectomized female sacrificed 3/18/40 in acute insufficiency 11 days after operation). Qualitatively the basophils in the adrenalectomized animal showed marked degranulation.

Chromophobes are most numerous in the medial third; basophils, more evenly distributed, reach peaks at either edge of the medial third; acidophils are most numerous laterally.

[1] In cooperation with the Department of Physiology, The Ohio State University.

183. Inclusion bodies voided from nerve cells and embedded in walls of blood vessels. James W. PAPEZ, Zoology Department, Cornell University.

Neurons affected by inclusion bodies tend to degenerate and release the inclusion bodies in clusters into the intercellular substance. The inclusions migrate to small blood vessel (veins) and become embedded in cytoplasm of connective tissue of outer wall of vessel forming vascular cuffs. Here they undergo degeneration, release their lipid, and a sclerotic patch is formed in the wall of the vessel encroach-

ing on the lumen (see demonstration D 2). When inclusion bodies migrate along outer walls of larger cerebral vessels, numerous sclerotic patches may be formed on the vessels, typical of cerebral arteriosclerosis.

In some instances the inclusion bodies enter the small blood vessels and become attached to the red blood corpuscles which swell to a spherical shape and stain darkly with cresyl violet.

184. Protargol used with the silver gelatin stain for nerve fibers. Anthony A. PEARSON, Department of Anatomy, University of Oregon Medical School.

The silver gelatin stain is often improved by a preliminary treatment with protargol. The method is outlined as follows:

Fix tissues in formalin, Bouin's fluid, or in any of the usual fixatives for silver stains. Imbed, cut, and remove the paraffin in the usual manner.

Place sections in a .5% solution of protargol for 8 to 10 hours at 37°C. Add copper as in the Bodian stain.

Water adjusted approximately to a pH of 4 to 4.5 should be used for the solutions in the following steps until the sections are placed in gold. This adjusted water can usually be prepared by adding 1 to 5 cm^3 of 1% citric acid to 1 liter of distilled water.

Drain off the protargol solution and place the sections in 2% silver nitrate for about 45 minutes in a dark oven at 55°C. This step may sometimes be omitted.

Place slides in a solution containing 10 cm^3 of 2% silver nitrate and 40 cm^3 of 3% gelatin. Place in a warm bath (about 55°C.) and add 4 cm^3 of 1% hydroquinone. Agitate this solution gently until the sections turn a tobacco brown.

Wash in warm water. Tone lightly in 1% gold chloride. Wash and place in 1% oxalic acid until the sections turn slightly purple.

Wash, dehydrate, clear, and mount sections in the usual manner.

185. Luteinization of theca interna cells in the ovaries of albino rats. E. Stanley PEDERSON and John S. LATTA, Department of Anatomy, College of Medicine, University of Nebraska.

In histological examination of rat ovaries in various stages of the sex cycle several examples of thecal luteinization in the walls of unruptured vesicular follicles were found. Two of these follicles were classified as simple follicular cysts, the remainder as atypical examples of atresia.

The hypertrophied thecal cells formed quite compact masses in juxtaposition to the stratum granulosum, which was in various stages of disintegration. Usually such masses were found only in one portion of the theca, however in some instances the masses had completely encircled the follicle.

The hypertrophic cells are histologically identical with lutein cells of the rat ovary. Transitional stages between typical theca cells and "theca lutein" cells were obvious.

In some instances, in which the stratum granulosum had entirely disappeared, interstitial cells (theca) were found which were apparently in the process of transformation into "theca lutein" cells.

From the material observed thus far it seems probable that this phenomenon occurs in unruptured follicles early in diestrum.

186. Some observations on the surface of some epithelial cells. Paul H. RALPH, Department of Anatomy, The Ohio State University. (Introduced by R. A. Knouff.)

By the use of the phase-difference microscope, it can be seen that the surface of desquamating cells from some stratified squamous epithelia (cheek, heel, tongue et al.) exhibits a complex pattern of tiny ridges or thickenings. These ridges exhibit variety and individuality equal to that of human finger prints. At the point where 2 epithelial cells are in contact the pattern of ridges of 1 fits into the grooves between the ridges on the surface of its neighbor. They may be seen only with the phase-difference microscope. There appear occasionally suggestions that they may be related in some way to the tonofibrils found in cells deeper within the epithelium. However, the resemblance of these patterns to striae developed in drying protein and collodion films suggests the possibility that this is merely an effect of desiccation.

187. Bulbar structures involved in swallowing. R. RHINES, Department of Anatomy, Northwestern University Medical School.

In cats under light nembutal anesthesia, the medulla was explored for swallowing responses by the Horsley-Clarke method. Rhythmic swallowing was elicited during stimulation of the dorsal part of the medulla in, and immediately adjacent to, the tractus and nucleus solitarius. Stimulation was not effective elsewhere in the bulb except along the intramedullary course of the glossopharyngeal and vagus nerves. Caudally, the excitable areas converged to the midline in the region of the commissural nucleus. No rhythmic swallowing was produced by stimulation caudal to this level.

Because of the close proximity of the tractus and nucleus solitarius and the adjacent gray, it was impossible to place precise enough lesions to designate the specific structure responsible for rhythmic swallowing. However, the extension of excitable points to the level of the commissural nucleus, where the tractus solitarius has almost disappeared, suggests that the response obtained was not solely due to activation of primary afferents.

Dysphagia is commonly observed clinically in instances of bulbar involvement, and has usually been attributed to injury to the nucleus ambiguus. The demonstration of supranuclear structures concerned with this activity suggests that they should be scrutinized also in determining the neuropathological basis for impairment of the swallowing reflex in affected cases.

188. Effects of pressure on the uterine eggs of Rana pipiens. Roberts RUGH and Douglas MARSLAND, New York University.

Previously the authors found that the mature spermatozoön of Rana pipiens was not appreciably affected by moderate hydrostatic pressure of 8,000 lbs./sq. in. for periods up to 30 minutes. Similar tests have been made recently on the uterine eggs, all of which are known to be in metaphase of the second maturation division. In each experiment 1 egg-filled uterus of an ovulating female was used as a control while the other similar uterus was subjected to hydrostatic pressure of

8,000 lbs./sq. in. for 3, 10, and 30 minutes. Immediately after compression the eggs from both uteri were fertilized with normal spermatozoa.

Some entire batches of eggs appeared to be undamaged by the pressure. They fertilized and developed normally. However, other batches of eggs displayed widespread cytolysis, some immediately after decompression. Other batches showed a low percentage of cleavage, death at gastrulation, and a high percentage of abnormalities during later development. These results indicate a varying susceptibility to compression damage in the eggs from different females. It appears that in this egg, as in previous observations, the cells are more susceptible to compression damage during periods when movements (e.g., mitotic) are occurring. Moreover, a preliminary test indicates that the germinal vesicle of the ovarian egg is quite resistant to pressure damage.

188a. Persistent pupillary membrane and hyaloid artery in the postnatal rat and mouse. Meredith N. RUNNER,[1] Roscoe B. Jackson Memorial Laboratory. (Introduced by Katharine P. Hummel.)

Although persistence of the pupillary membrane and hyaloid artery is common in man there is practically no evidence of an hereditary basis for this defect. The eyes from 6 strains of inbred mice and 2 strains of inbred rats were examined prior to opening of the eyelids. The hyaloid artery, vascular lenticular capsule and pupillary membrane were present in all eyes examined. Their major portions disappeared about the time that the eyes opened. A series of vascular injected, cleared and mounted eyes depicts the embryological basis for one of the most common anomalies in the eye of man.

One strain of Jackson Laboratory rats showed 100% persistence of an incomplete pupillary membrane throughout life and 100% persistence of the hyaloid artery for the first week after the eyelids opened. The pupillary membrane in the newly opened eyes changed from an almost complete, vascularized membrane to a few vessels at the age of 2 months. A similar but less pronounced situation was found in one strain of mice (Bagg alb. C). Here both the extent of the membrane and frequency of persistence was less. In contrast to the situation commonly seen in man, the persistent pupillary membranes in rats and mice are vascularized structures. They consist of one of twelve vascular loops derived from the second and third arcades of the anterior vascular capsule.

[1] Finney-Howell Fellow.

189. Some observations on the maturation of the histomorphology of human bone. Elbert B. RUTH, Department of Anatomy, University of Cincinnati, and Emil W. HAURY, Department of Physical Anthropology, University of Arizona.

The histomorphology of the femurs of a series of human fetuses, infants, children, young adults, and adults is described. The ages of the subjects range from the 3-month fetus to the 93-year-old adult. Most of the femurs are from the collection of American Indian skeletons in the Arizona State Museum. Ages given are only approximately as estimated by the physical anthropologist, with a few exceptions where the age is known. The development of the laminar type of bone

characteristic of the fetus and infant is described, followed by the origin and development of Haversian canals between the first and second years. The characteristic circumferential and longitudinal vascular spaces of the fetus and infant are followed through their metamorphosis into the Haversian type of vascular channels, and these in turn are described as the histomorphology of the femur begins to approximate that of the adult at about the eighth year. The circumferential interlamellar canals and the relatively wide band of external circumferential lamellae that appears to be charcteristic of the bones of children and young adults apparently remains until the eighteenth year, becoming less complete or absent in older bones.

190. An analysis of variations in the arteries and veins of the bronchopulmonary segments of 50 right upper lobes. J. Gordon SCANNELL, Department of Anatomy, University of Minnesota. (Introduced by Edward A. Boyden.)

The 3 segmental arteries — apical (A^1), anterior (A^2) and posterior (A^3) — arise exclusively from the anterior side of the hilum in only 8% of specimens. In the remainder, the lobe is supplied from both the anterior and interlobar sides. Anterior arteries are grouped into a commonly bifurcate *truncus anterior* (88%) or into a *truncus superior* and a *truncus inferior* (12%). A^1 is always anterior in origin. A^2 and A^3 may be either anterior or interlobar (the ''recurrent'' and ''ascending'' arteries of Appleton) or both. A^2 is wholly recurrent in 58%, mixed in 42%; A^3 is wholly recurrent in 18%, ascending in 12% and mixed in 70%, but its ramus A^3a is wholly recurrent in 60%, and A^3b is wholly ascending in 56%.

Of the 3 veins — Apical-anterior (V^1), inferior (V^2) and posterior (V^3), the last is the most variable. In 50% it is formed by confluence of interlobar and central divisions, but the posterior vein may be wholly central in position (28%) or wholly interlobar (16%). In the remaining 6%, the 2 divisions empty separately — the interlobar coursing either posterior to the pedicle of the middle lobe, to enter the superior pulmonary, or posterior to the pedicle of the inferior lobe to reach the inferior pulmonary vein. In 52% of cases the interlobar division receives a tributary from the superior segment of the lower lobe.

191. The fundamental transverse arrangement of cross striae in myofibrils of striated muscle. Carl Caskey SPEIDEL, School of Anatomy, University of Virginia.

Recently the ''actin-myosin screw theory'' of myofibril structure has been proposed by Szent-Gyorgyi and Gerendas ('45). It is claimed that as a striated muscle fibril is rotated, the cross striae shift in spiral fashion like the thread of a screw, the shift being equal to one sarcomere length as a fibril is rotated 180 degrees. Szent-Gyorgyi has elaborated a detailed theory of muscle contraction based on this concept.

To check this screw theory, I have examined an exceptionally favorable type of striated muscle from the pharynx of the sea-spider, Anoplodactylus lentus. A typical fiber is a short, slender, single-celled structure containing about a dozen coarse myofibrils. The sarcomeres are of large size, each measuring about 9 micra in length in resting condition.

A simple technique was devised which permitted very satisfactory observations on whole fibers as these were rotated various amounts from 0 to 360 degrees. The muscle nucleus afforded a convenient fixed landmark. Dozens of fibers were carefully examined, attention being focussed in each case on individual myofibrils as rotation proceeded.

These observations clearly showed that there was no spiral or screw-like shift of cross striae within individual myofibrils. On the contrary, throughout the rotation the transverse arrangement of Q, J, and Z discs within each myofibril was maintained at the same level.

Similar results were obtained with shrimp (Palaemonetes) muscle.

192. Response of germinal epithelium of the mouse to thyroxin in the ovarian capsule. Kathryn STEIN and Jewel QUIMBY, Department of Zoology, Mount Holyoke College.

A single injection of thyroxin (0.005%) into the left ovarian capsule in a series of 14 albino mice invariably caused inhibition of proliferation in the germinal epithelium within a period of 24 or 28 hours after injection. Controls consisted of the right ovaries of the experimental mice and 6 animals which were either untreated or received systemic injections. Colchicine was given to all animals to facilitate counting mitoses. The thyroxin was injected in sesame oil or in Ringer's solution; control right ovaries were treated with the corresponding vehicle minus the hormone. Sesame oil stimulates proliferation but to a much lesser degree when accompanied by thyroxin. Ringer's treated right ovaries showed approximately the same number of mitotic figures as were found in normal untreated animals. Immature females had the largest average number, estrous females the next, and diestrous the least. The decrease in proliferation in the germinal epithelium of the left ovaries due to the thyroxin injections was as high as 70% in individual mice, averaged around 50%, and was never less than 30% (2 animals). The effect of thyroxin is interpreted as due to the direct action of the hormone on the cells because of the short time interval following injection and the localization of the effect to the thyroxin treated ovary in each mouse.

192a. Embryonic bone transplants in drill hole defects of adult long bones. Charles STEINMAN, Department of Anatomy, Vanderbilt University. (Introduced by Sam L. Clark.)

To study the process of bone repair, and the influence of embryonic bone upon this process, 22 tibiae of adult rabbits were exposed, stripped of periosteum and a groove made in each. Into each of 14 defects were placed small bone fragments of 18-day rabbit embryos. Nothing was put in the remaining 8 defects.

The development of firm exostotic nodules was noted over each defect containing embryonic bone transplants but not in the controls.

Microscopic sections of the controls revealed that the defects were first filled by blood and fibrinous clot, which was replaced by fibrous and granulation tissue by the seventh day. New bone from periosteum and endosteum bridged the gap by the twenty-eighth day and then remodeling of the cortex took place.

Sections through the region of the defects with transplants revealed the absorption of the embryonic bone by the seventh day, and the presence of many centers

of proliferating cartilage in the fibrous callus. By the twenty-first day masses of cartilage filled the defect, extended into marrow cavity, and protruded onto the surface. The cartilage was slowly replaced by new bone and the gap was bridged by seventieth day. Some cartilage was present after 150 days in the exostosis.

While bone defects are commonly repaired by intramembranous bone formation, those filled with embryonic bone, showed cartilage proliferation, and endochondral bone formation.

193. *Primitive carotid intersegmental arteries and the formation of the basilar in rabbit embryos injected while alive.* Leon H. STRONG, Department of Anatomy, University of Michigan.

When the primitive internal carotid of the first aortic arch stage reaches the brain at the diencephalon to anastomose with the primary superficial plexus of the brain, it forks into a rostral and dorsal branch. The dorsal may be considered the first intersegmental. Just caudal to this is a second intersegmental supplying the trigeminal ganglion. This forks into a caudal and rostral branch. Intersegmentals behind this do the same. A ventral line is formed by anastomosis on either side, a longitudinal trunk from diencephalon to the last caudal segment, the primitive arterial tract of Adamkiewicz. With the rapid enlargement of the brain the primitive carotids move lateral and dorsal. The bases of their intersegmentals are carried lateral. With the degeneration of the first 2 aortic arches, the interarch segments which persist back to the third arch, now the internal carotid, give off intersegmentals for some time before these atrophy and disappear. But those intersegmentals from the aorta, serially continuous caudally, do not. The cranial ends of the 2 arterial tracts, by anastomosis and enlargement form a basilar trunk. This basilar segment of the arterial tract is developed from anastomoses between the carotid intersegmentals only. The basilar is not an extension forward from the vertebral arteries as previously supposed.

194. *Establishment of differences in susceptibility to audiogenic seizures of 5 endocrinic types of mice.* E. M. VICARI, R. B. Jackson Memorial Laboratory.

Five inbred strains of mice previously reported as different in the histochemistry of their adrenal glands were observed with respect to their susceptibility to audiogenic seizures followed by death. These strains are: dilute brown, albino-A, extreme dilution, C3H, c57 black. Both sexes in equal numbers were used involving 571 individuals. Ages tested were from 15 to 60 days and 3 to 9 months. Audiogenic convulsions were brought on by ringing of an electric bell.

Results show that the dba and black strains were most susceptible at 30 days, 78% and 83% individuals had seizures of which 67% and 58% died. But the blacks at 35 days dropped to 8% with 90% deaths while the dba maintained its high susceptibility with 44.4% deaths. Neither strain showed any susceptibility at ages 3 to 9 months. The extreme dilution strain showed a high susceptibility at age 55 days with 83% seizures, 100% recovery; at 7 to 9 months 100% seizures and recovery. The albino-A had no convulsions at any age under 3 months. At 5 months 28.5% had seizures of which all recovered. The C3H strain, all under 3 months tested, showed no convulsions whatsoever.

The dba and black strains are less susceptible with age while the extreme dilution and albino-A strains are more so with age. The C3H are unaffected.

195. *The rate of renewal in man of the water and sodium of the amniotic fluid as determined by tracer techniques.* G. J. VOSBURGH, D. B. COWIE, L. M. HELLMAN, W. S. WILDE, and L. B. FLEXNER, Department of Obstetrics, Johns Hopkins University and Hospital and the Departments of Terrestrial Magnetism and Embryology, Carnegie Institution of Washington.

The amniotic fluid was considered a relatively stagnant body fluid until the availability of isotopes permitted measurement of the rate of renewal of its constituent substances. Such measurements in the guinea pig showed that the water of the fluid is replaced about once an hour whereas the rate of replacement of sodium is about 50 times slower.[1]

We have measured water and sodium transfer to the human amniotic fluid using deuterium oxide and radioactive sodium as tracer substances. The observations with sodium were made on 20 women, and with water on 5 women on whom hysterotomies were performed. We have found that on the average 0.345 ml of water is transferred to a ml of amniotic fluid per hour; i.e. the water of human amniotic fluid is turned over once every 2.9 hours. The amniotic fluid of man contains 2.8 mg sodium per ml[2] and sodium is transferred at an average hourly rate of 0.137 mg per ml; hence the sodium of the fluid is turned over once every 20.5 hours. It is evident that the membranes which are traversed by substances reaching the amniotic fluid from the maternal circulation are far more permeable to water than to sodium and that the water of the amniotic fluid is replaced with astonishing rapidity.

[1] Flexner, L. B., and A. Gellhorn. Am. J. Physiol., *136*, 757, 1942.
[2] Needham, J., Chemical Embryology. Cambridge, England. 1931.

196. *On the staining of fat and fatty substances with azo dyes of the Sudan series in aqueous solution.* Harold L. WEATHERFORD, Department of Anatomy, Harvard Medical School.

It is well known that certain substances insoluble, or soluble with difficulty, in water can be dissolved in aqueous solutions of other substances. Neuberg has called this phenomenon hydrotropism — and supposes that a weak addition-compound is formed between the substance dissolved and the dissolving or hydrotropic substance. The ideas of Neuberg have been applied to several members of the Sudan series of azo dyes in an attempt to produce aqueous solutions that will stain fat and fatty substances in tissues. More than 80 potential hydrotropic agents have been tested and a large number of them, when compared with 70% alcohol are found superior as solvents for Sudan dyes. Four criteria are required of hydrotropic solutions of these dyes: (1) they should stain fat or fatty substances more clearly than a solution of the same dye in 70% alcohol; (2) there should be no "bleeding" of the dye into the tissue; (3) they should produce no deleterious effects on the tissue; (4) the methods finally recommended should be practical for daily use. The report now in preparation contains a full discussion of hydrotropism, the principles involved and their applicability.

197. *Progress of studies designed to determine whether the fetal hypophysis produces hormones that influence development.* L. J. WELLS, Department of Anatomy, University of Minnesota.

Fetal rats with placental circulation intact were deprived of their hypophyses by decapitation. Of the 17 males that survived for periods of 28 to 102 hours before severance of the umbilical cord near term, 5 were given a series of 5 subcutaneous injections of equine gonadotropin (Gonadogen). Injections were made twice daily. In autopsying littermate controls, they were decapitated and their headless bodies weighed. Stained serial sections of adrenals and of sex glands were studied microscopically and then used in determining the volume of these organs (paper-weight method).

While the data are too few to show whether the fetal hypophysis produces adrenotrophin, they suggest that it actually does. Thus in the 2 uninjected fetuses deprived of their hypophyses for the longest periods (102 hours, 77 hours), the volume of the adrenal, in cubic millimeters per gram of headless body weight, was less than half that of littermate controls (39%, 48%).

As to whether the fetal hypophysis produces gonadotrophin, the preliminary observations do not warrant a definite conclusion. The volume of the reproductive glands, when expressed as percentage of body weight, was similar to that of controls (testis, seminal vesicle, bulbo-urethral gland). However in all injected fetuses the interstitial cells of Leydig were much larger and far more numerous than those of "hypophysectomized" controls.

Preserved organs of 6 "hypophysectomized" female fetuses require additional study.

198. *Reaction of damaged hepatic parenchyma of mice to vital staining.* W. Lane WILLIAMS, Department of Anatomy, University of Minnesota.

Ten male albino mice received daily subcutaneous injections of 0.2 cm^3 of trypan blue (0.5% aqueous solution) and either 0.03 cm^3 of chloroform or 0.04 cm^3 of carbon tetrachloride for 4 to 7 days. The mixtures of the latter contained 1 part of carbon tetrachloride or chloroform in 4 parts of mineral oil.

The type of hepatic parenchymal injury characteristically produced by chloroform or carbon tetrachloride resulted. Many foci of liver cells showed cytoplasmic vacuolation and eosinophilia, and either nuclear pyknosis or absence of the nucleus. The cytoplasm of the hepatic epithelium in such areas of injury was diffusely stained or colored by trypan blue. The dye was present in these cells as an amorphous suffusion and not in the form of granules and particles common to macrophages. Identical methods of histological technique demonstrated no deposition of dye within morphologically normal hepatic epithelium either in these mice or in animals not treated with carbon tetrachloride or chloroform. The dye in the cytoplasm of damaged liver cells was easily removed by subjecting the sections to water or aqueous solutions of alcohol whereas the dye within macrophages was relatively resistant to the same procedures.

The findings demonstrate that necrotic and/or damaged liver cells react to parenterally administered dye in a manner similar to certain other epithelial and non-epithelial cells.

DEMONSTRATIONS

(The numbers indicate the order of the demonstrations on the program of the meeting.)

D 32. Follicular atresia in the ovaries of newts injected with diethylstilbestrol. A. Elizabeth ADAMS, Department of Zoology, Mount Holyoke College.

The ovaries of adult Triturus viridescens given total doses of 5.0–7.0 mg of diethylstilbestrol [1] in sesame oil intraperitoneally over a period of 78–108 days were compared with those of controls injected with sesame oil only. At autopsy, the ovaries of the stilbestrol-injected newts often looked cystic and oil-filled. Only non-yolked oocytes were present together with masses of tissue resulting from the resorption of heavily yolked oocytes. The ovaries of the oil-injected controls were noticeably smaller, but also often looked as if they contained oil. They consisted of small non-yolked oocytes and typical pigmented corpora atretica. The average ovarian weight in alcohol for the 10 stilbestrol-injected newts was 224.9 mg and for the 10 oil-injected controls, 42.1 mg.

Histological examination revealed oogonia and non-yolked oocytes in varying sizes and numbers in the ovaries of both groups. The most striking difference between the groups was the appearance of the corpora atretica. Those in stilbestrol-injected newts were in general very much larger than those in controls and consisted largely of epithelioid cells, containing varying amounts of pigment and were slightly vascularized. Yolk granules were sometimes present, depending on the age of the corpus atreticum. In the oil-injected controls, the corpora atretica showed the typical vacuolated, pigmented and well-vascularized condition and the size characteristic of the urodele yolked oocyte undergoing resorption after the spawning season, or hypophysectomy or starvation.

[1] Diethylstilbestrol supplied through the courtesy of Dr. C. W. Sondern, George A. Breon and Co., Inc., Kansas City, Missouri.

D 38. Staining reactions with the Feulgen and dinitrophenylhydrazine reagents. S. ALBERT, Department of Anatomy, McGill University. (Introduced by C. P. Leblond.)

The distribution of the Feulgen and dinitrophenylhydrazine reactions was examined in formalin-treated normal and cancerous tissues of mice and rats of various ages. These techniques enabled a cytological study of these reactions which was especially clearcut in the case of the dinitropheylhydrazine reaction. The effects of strain and age differences will be especially emphasized.

D 27. The transparent chamber technique in the mouse in the study of vascular physiology and tissue growth. Glenn H. ALGIRE, National Cancer Institute, National Institute of Health, United States Public Health Service, Bethesda.

The transparent chamber technique as adapted to the mouse is presented by means of microscopic demonstration of the living tissues, photographs and models illustrating the technique, graphs, and photomicrographs to show applications in quantitative studies on vascular physiology and tissue growth, with special reference to cancer.

D 30. Dermal characteristics in the presence of basal cell epitheliomas of human beings and after regression following treatment with tissue extracts. Joseph C. AMERSBACH, Elsie M. WALTER, and George S. SPERTI, Skin and Cancer Unit of the New York Post-graduate School and Hospital and the Institutum Divi Thomae. (Introduced by Rucker Cleveland.)

Biopsy specimens obtained from 47 patients with basal cell epithelioma prior to and following treatment with human and beef spleen and liver extracts were fixed in Bouin's fluid and stained with hematoxylin and eosin. The lesions ranged as large as 4 cm in longest dimension. Some were as recent as 1 month in development, others had existed for several years. The occurred chiefly on the cheek, forehead, nose, and base of the scalp. The age range of the patients was from 33 to 82 years. Intradermal infiltrations using 0.5 to 1.0 cm^3 of tissue extracts (1.0 $cm^3 \cong$ to 1–15 gm tissue) were made weekly about the lesions, as few as 3 and as many as 20 treatments being required for complete regression. The detailed clinical report appeared in the "Archives of Dermatology and Syphilology," 54, 119, August, 1946, and a second is in press in the same Journal.

Study of the sections revealed a variety of histologic characteristics including some basophilia of the connective tissue components. There was an increased number of various kinds of connective tissue cells in specimens obtained before as well as after treatment, when these were compared with sections of normal skin fixed and stained in the same manner. The nature of the process of regression is undetermined.

D 42. Leukocyte migration into and through the epithelium of the trachea in the mouse.[1] Warren ANDREW and Marjorie R. BURNS, Department of Anatomy, Southwestern Medical College.

Slides and photomicrographs are presented to illustrate some of the phenomena of migration of leukocytes into and through the epithelium of the trachea. It has long been known that such a phenomenon occurs in the normal animal but little attention has been paid to the details. A variety of cell types are seen to enter the epithelium in the present material. They are primarily lymphocytes, but many polymorphonuclear neutrophils are present, in contrast to such migration in the intestine, where the cells are practically entirely lymphocytes. The presence of large vacuoles about cells of whatever type which have entered the epithelial layer is a conspicuous feature. While most of the leukocytes here are intercellular in position, they frequently cause a very great deformation of the epithelial cells and figures are seen suggestive of an intracellular penetration in some cases. The leukocytes frequently are greatly elongated and may be mistaken for modified epithelial cells in sections. Changes in lymphocytes such as pycnosis and fragmentation, common in the intestinal epithelium, are rare in the trachea but do occur in some instances.

[1] This study was supported by grants from the Winthrop Chemical Company and the John and Mary R. Markle Foundation.

D 18. A simple method for obtaining photographic and cinematographic records of embryos and other small objects. Leonard-F. BÉLANGER, Department of Histology and Embryology, School of Medicine, University of Ottawa, Canada.

The necessity of securing records of the general aspect of human embryos which are to be sectioned serially, has prompted the development of the following apparatus. A Cine-Kodak Titler is fixed to a wooden support which permits its use in a mobile vertical position. For photography, a 35-mm camera is secured to the Titler with a mounting block. Records can be obtained this way of objects up to 60 mm in length and 20 mm in width and thickness. The object is easily framed and photographed along with 2 rulers at right angles to one another and a strip of paper bearing the necessary data. The film strip can be studied with a microfilm reader; it can be projected or an enlarged print, the size of a filing card, can be made of it, permitting accurate longitudinal and cross measurements. For cinematographic studies of the living chick, frog, fish and invertebrate embryos, a standard movie camera is substituted for the still camera and its support in the apparatus. Experiments can be performed while the embryo is easily maintained within the field of the instrument.

D 17. The developmental stages and anatomy of Xenobus laevis, African horned toad. T. W. M. CAMERON and Mrs. CAMERON, Institute of Parasitology, McGill University. (Introduced by S. M. Friedman.)

This toad was introduced to science by Professor Hogben and his colleagues in South Africa. It is bred extensively for use in pregnancy diagnosis and hormone research.

This demonstration shows the various stages of development and the anatomy of the living animal from the egg to the 2-year-old sexually mature toad.

D 40. Microscopic demonstration of neurosomes in skeletal muscle following atrophy of disuse. Eben J. CAREY, Leo C. MASSOPUST, Walter ZEIT, and Eugene HAUSHALTER, Department of Anatomy, Marquette University School of Medicine.

(See abstract of paper from platform.)

D 28. A comparison of growth and differentiation of avian femur rudiments on clots formed from embryo extract and plasma with those grown on clots of partially or completely known constituents. Esther CARPENTER and Ursula ROTHFELS, Department of Zoology, Smith College. (Introduced by Esther Carpenter.)

As controls, femur rudiments of 6-day chick embryos were grown by the watch glass method (Fell, '29) on the surface of clots composed of equal amounts of adult fowl plasma and 10% embryo extract from 10-day chick embryos.

For the experimental cultures the following combinations were tried: (1) White's ('46) synthetic medium for animal tissues to which washed agar had been added to produce a concentration of 0.75%; (2) a clot composed of bovine fibrinogen (Armour), bovine thrombin (Upjohn) and White's synthetic medium; and (3) a clot composed of fibrinogen, thrombin and embryo extract.

In cultures in which the synthetic nutrient replaced the embryo extract during the first week there was no growth in length, pycnotic nuclei were numerous and the femur rudiments were slightly opaque. After 2 weeks fewer pycnotic nuclei were present, the rudiment had increased in length and improved somewhat in shape. No bone was observed.

Femur rudiments on clots containing embryo extract but with the plasma replaced by bovine thrombin and fibrinogen, grew and underwent morphogenesis as well as those with plasma. The bone deposited was thinner, however, and the cartilage cells less vacuolated.

D 51. A cell type possibly responsible for facultative salt excretion in the gills of Fundulus heteroclitus (a euryhaline fish). D. Eugene COPELAND, Biology Department, Brown University. (Introduced by A. B. Dawson.)

A cell type is demonstrated that shows consistent cytological pictures, one specific to fresh water and the other to sea water (or concentrated NaCl). The cells are located in the gill filament and elongated in most cases to reach from the circulation to the surface of the filament. Regaud preparations show a rich profusion of apparent mitochondria. Prolonged osmication (Mann-Kopsch procedure for 1 month in 2% osmic tetroxide at room temperature) causes a specific blacking of these cells that is strongly retained after all other elements have been decolorized. Behavior is gauged by the presence of a vesical at the distal end of the cell in sea water and absence of the vesicle in fresh water. Mucicarmine and thionine tests are negative for mucus in this cell type.

D 36. Selective impregnation of cells of the basophilic series of the anterior pituitary gland of the rat and chicken: their early recognition in the fetal rat and embryonic chicken. Alden B. DAWSON, The Biological Laboratories, Harvard University.

In fully differentiated anterior pituitary glands of the chicken prepared by Bodian's protargol method without gold toning, typical basophiles and smaller cells, interpreted as partially differentiated basophiles, are deep black. Acidophiles of both types are reddish-brown and inactive chromophobes are bright yellow. In the rat only regular basophiles and less differentiated basophiles react strongly with silver. In all these cells of the basophilic series the elements reacting with silver appear as discrete spherical bodies and do not seem to be identical with the diffuse type of granulation demonstrated in basophiles with anilin blue. The history of the cells, blackened specifically with protargol, has been followed in progressively younger animals to determine the time of their first appearance. They were first found in the embryonic chick on the eighth day of incubation and in the fetal rat on the sixteenth day of gestation. It has not been possible to identify these argentophile cells at such early ages by the selective staining methods usually applied to the pituitary gland. Thyroidectomy cells of the chick, induced by thiouracil treatment, have little affinity for silver but a ring of blackened granules frequently surrounds the negative image of the Golgi apparatus. In the rat modified basophiles — thyroidectomy cells (thiouracil treatment) and castration cells — gradually lose their ability to react with protargol.

D 33. A histological study of eye abnormalities in the C57 black strain of mice. Patricia DOUGLASS and W. L. RUSSELL, Jackson Memorial Laboratory. (Introduced by Katharine Hummel.)

Non-genetic eye abnormalities occurring spontaneously in the C57 black strain range from corneal opacity to eyelessness. The abnormalities may be present in both eyes or only one, much more commonly the right, and are more frequent in females than in males.

A considerable variation in kind and degree of defects is shown by histological examination. The lower grades of abnormality are characterized by opacities of varying size, waviness and invagination of retinal layers sometimes accompanied by proliferation of the cells in one or more layers with or without invasion into adjacent layers. Fusion of the cornea with the choroid or lens, and of the retina with the choroid often occurs, also proliferation and disorganization of the choroid. In more extreme abnormalities the eyeballs are very small and consist of partially differentiated but disorganized connective, pigmented and retinal tissues imbedded in muscle. The most extreme cases are characterized by the presence of only muscle and lacrimal gland in the orbit.

The fact that the whole range of abnormalities fits into a single pattern of defects suggests that the C57 black strain is prone to irregularity in one particular phase of eye development.

D 47. The anatomical distribution of the ductules and islets of the rabbit's pancreas in alloxan diabetes. G. Lyman DUFF, Department of Pathology, McGill University. (Introduced by B. Kropp.)

The demonstration of the ductular ramifications in the normal rabbit pancreas is both difficult and unsatisfactory. However, it is possible to obtain a selective hydropic change of the epithelial cells of the ductules and islets of Langerhans by experimental means which renders their anatomical distribution and relationships apparent.

Moderate to extreme hydropic degeneration of the pancreatic ductules and islets was observed in rabbits rendered diabetic by alloxan when the diabetic state had persisted for several months. Such changes have not hitherto been described in alloxan diabetes in the rabbit. The earliest appearance of these alterations was at the end of 45 days after the injection of alloxan and hydropic changes were never absent after 90 days providing that the average fasting blood sugar level during the experiment had been 303 mg % or higher. The hydropic state of the ductules and islets persisted without histological evidence of any further change in association with more or less severe diabetes lasting for periods up to 1 year. The alpha cells of the islets remained histologically normal but appeared to be increased in number. Preliminary observations demonstrated that the hydropic degeneration of both ductules and islets is reversible by adequate treatment of the diabetic state with insulin.

D 21. Two unusual anomalies in the same cadaver (Negro male, age 85 years). John C. FINERTY and Mildred TROTTER, Department of Anatomy, Washington University.

I. Persistent right ischiatic artery. The anomalous artery is a direct continuation of the anterior division of the hypogastric artery and passes through the greater sciatic foramen closely associated with the tibial division of the sciatic nerve. After sending branches to posterior thigh muscles the vessel extends inferiorly as the popliteal artery with normal course and relations. A typical, but small, femoral artery is present and anastomoses with the popliteal artery.

II. Hourglass bladder. The urinary bladder is divided by a horizontal septum into 2 chambers of approximately equal capacity. The upper chamber is somewhat to the right of the midline and posterior to the lower chamber, which is in the usual position in the pelvis; these are connected by a centrally located, circular orifice 1 cm in diameter. The ureters terminate in the lower chamber on the extremities of a well-marked transverse ridge. The right ureter, larger than the left, traverses part of the septum. The remaining angle of the vesical trigone is represented by a typical urethral orifice.

D 34. The effect of oral administration of vitamin A on the expression of the recessive gene "rhino" in the mouse. F. Clarke FRASER, Department of Genetics, McGill University. (Introduced by J. C. B. Grant.)

The first visible abnormality in the skins of homozygous rhino mice is a hyperkeratosis of the epidermis and follicle neck walls at the time when active growth of the first hair generation ceases (14 days), which is associated with a widening of the hair canals and a subsequent falling out of the hair coat. This is followed by the development of cysts in the hair-canals, the sebaceous glands and the follicle-ends, a thickening and wrinkling of the epidermis and complete suppression of further hair growth.

The skins of mice fed massive oral doses of vitamin A (of the order of 500–1000 International units every other day) for several months are much thinner and less wrinkled than those of litter-mate controls. Preliminary histological examination shows a reduction in the number and size of the sebaceous gland and follicle end cysts, and signs of abortive attempts at new hair growth in the treated mice.

D 29. Methods for the experimental investigation of cardiovascular-renal dynamics in the rat.[1] S. M. FRIEDMAN and C. L. FRIEDMAN, Department of Anatomy, McGill University.

a. Previously described methods for the study of renal function in the rat have been simplified. The present procedure makes use of sodium-p-amino hippurate as a test substance to measure tubular excretion and renal blood flow, while inulin is used to estimate glomerular filtration. The demonstration consists of graphs and charts illustrating the development of the method and its final form.

b. A method for the direct reading of heart rate and heart sounds on the Cathode Ray tube is demonstrated.

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[1] This work was supported by a grant from the Life Insurance Medical Research Fund.

D 4. Boutons terminaux. W. C. GIBSON, Montreal Neurological Institute. (Introduced by C. P. Martin.)

Synaptic endings in the spinal cord, sympathetic nervous system, cerebellum, basal ganglia and cerebrum will be demonstrated by slides stained by block impregnation or frozen silver methods. At present it appears that the *boutons terminaux* stain with much greater facility in the spinal cord, but the development of modifications for cortical material may show the same richness of synaptic endings in that area.

The method of preference for staining synaptic endings would seem to be Rio-Hortega's double impregnation technique, employing a preliminary staining with dilute silver nitrate followed by silver carbonate. The chief practical use of the method lies in determining the precise point at which a tract ends, as shown by its degenerating end-feet situated on the surface of the next neuron in the circuit. Criteria for determining the number of days following tract section, at which degeneration is best seen, have still to be worked out for cortex, cerebellum and basal nuclei.

D 9. An hypophysial portal system in certain amphibia, and nerve endings associated with neurohypophysial vessels. J. D. GREEN, Department of Anatomy, Wayne University. (Introduced by Gordon H. Scott.)

The hypophysis of Rana catesbiana is supplied by 2 large arteries from the main divisions of the internal carotid. These pass backward on the under surface of the hypothalamus in the pia-arachnoid. Just before they reach the gland each subdivides into a leash of arterioles and penetrates a small thickening of the neural stalk, which projects between the 2 bulging lobes of the pars intermedia, to form a rich capillary plexus within it. Blood issuing from this plexus is collected into from 1 to 4 large vessels passing backward on the under surface of the adenohypophysis, which is supplied by capillaries arising from them. Venous blood is collected into a large skein of veins issuing from the lateral aspect of the gland and discharging into the plexus of the saccus endolymphaticus. In Amblystoma tigrinum a somewhat similar arrangment occurs although the effluent channels from the neural stalk are diffuse. Though a hypophysio-portal system is not anatomically very apparent in this form, as stated by Roofe and by Craigie, a similar functional arrangement seems to exist. Similar observations have been made on Triturus torosus and Necturus. India ink preparations, dissections, and illustrations are demonstrated, and nerve endings associated with the neurohypophysial vessels are also shown.

D 31. Cytochemical evidence for the cessation of hormone production in the zona glomerulosa of the adrenal cortex during treatment with desoxycorticosterone. Roy O. GREEP, Harvard School of Dental Medicine, and Helen Wendler DEANE, Department of Anatomy, Harvard Medical School.

Ketosteroids (sudanophilic, Schiff-positive, acetone-soluble, birefringent, and fluorescent in formalin-fixed frozen sections) occur in both the zona glomerulosa

and the zona fasciculata of the rat's adrenal cortex. Previous experiments showed that the ketosteroids in the fasciculata might increase or decrease independently of any change of those in the glomerulosa. The former experiments suggested that the fasciculata contains corticosterone-like hormones, whereas the glomerulosa possesses the desoxycorticosterone-like steroids.

Two mg desoxycorticosterone-acetate (Ciba) was injected daily into both intact and hypophysectomized male rats. In both groups this treatment induced a slight atrophy of the adrenal but no alteration in the size of the thymus. Cytochemically the fasciculata was unchanged. In the glomerulosa, however, the ketosteroid reactions diminished gradually, with a detectable loss of fluorescence by 7 days, a reduction in sudanophilia and birefringence by 12 days, and a total loss of all the reactions employed by 28 days.

These results support the view that hormones concerned with salt balance, such as desoxycorticosterone, are produced in the zona glomerulosa of the adrenal cortex. When this type of hormone is provided in adequate quantity, the zone undergoes a disuse atrophy and ceases forming its normal secretion. A morphological duality of the cortex thus parallels the physiological and biochemical duality already recognized.

D 43. Comparative pulmonic alveolar size in man, cat, rabbit, monkey, guinea pig, goat, dog, baboon, rat and mouse.[1] W. Stanley HARTROFT, Banting and Best Department of Medical Research, University of Toronto. (Introduced by Charles C. Macklin.)

Microsections and photomicrographs illustrating differences in pulmonic alveolar size of man and 9 species of laboratory animals as seen in 25 μ paraffin microsections will be shown. The lungs were preserved in expansion either by vascular perfusion with fixative while in the unopened thorax, or by bronchial filling with the same, the degree of expansion being controlled by comparing certain pulmonic measurements with the corresponding pleural cavity dimensions. A wide variation in alveolar size was found, the largest being in man, and the others diminishing in order as in the title. Methods of measuring the alveoli so preserved will be demonstrated. Graphic comparisons of the arithmetic means of several thousand measurements will be presented. A summary of 50,400 measurements on 25,200 alveolar outlines will be given.

[1] This work was done in the Department of Histology and Embryology of the University of Western Ontario Faculty of Medicine, London, Canada.

D 37. A method of studying dry smears of blood with phase microscopy followed by staining techniques. Oliver P. JONES, Department of Anatomy, University of Buffalo and Oscar W. RICHARDS, Research Division, American Optical Co., Scientific Instrument Division.

Unstained dry smears of embryonic blood on cover slips were inverted on a drop or two of either Baker's formol-calcium or 10% formalin and ringed with

petrolatum. After studying cells with phase microscopy, it is possible to mark them for restudy with a bright field microscope following Wright's stain or Sudan black. Although bright and dark contrast phase microscopy may be used with Sudan black preparations, the latter combination brings out more detail than either technique alone.

D 22. Examples of anatomical specimens mounted in plastics. Otto F. KAMPMEIER and Thomas N. HAVILAND, Department of Anatomy, College of Medicine, University of Illinois.

Different kinds of anatomical preparations, such as dry and wet specimens, cleared specimens, celluloid, vinylite and Wood's metal corrosions, injections, dissections, gross sections, stained sections, whole mounts of fetuses, etc. are shown embedded in Castolite and Plexiglass.

D 35. Specialized argyrophilic (argentaffine?) cells in the anal canal of the rhesus monkey. Hadley KIRKMAN and Irene OW, Department of Anatomy, Stanford University.

As a normal epithelial component of the alimentary tract argentaffine cells are well known to be very widely distributed through all classes of vertebrates. They have been described repeatedly in simple columnar epithelia of all parts of the stomach and intestine, in the gall bladder, the pancreatic duct and the glands of Brunner. Reports of their presence in stratified epithelia, however, are rare.

In the stratified squamous epithelium of the ano-rectal junction there occurs, in 4 rhesus monkeys examined, a circular band of slender, bipolar cells. The cytoplasm of these cells contains argyrophilic granules similar, except for lack of polarized distribution, to those found in typical argentaffine cells. In many of these cells one or more fine, intracytoplasmic, argyrophilic fibrillae are recognizable, as is true also for many typical argentaffine cells of monkeys. While the cells are intimately related to fibers of the mucosal nerve plexus the relationship may be one of either continuity or contiguity. Many, and possibly all, of the cells appear to extend throughout the entire thickness of the epithelium. In the simple epithelium of the rectum a few transitions between them and typical argentaffine cells have been observed.

They are not the same as the tall, slender cells frequently observed in stratified epithelia elsewhere. They are absent in rats and hamsters and at cardiac-esophageal and cervico-vaginal junctions.

D 11. Reconstructions showing topography of fibers in medullary center of the human cerebellum. Wendell J. S. KRIEG, Institute of Neurology, Northwestern University Medical School.

Eight graphic reconstructions are demonstrated, representing the topography of fibers in the medullary center of the human cerebellum.

The appearance of the fibrous core of the cerebellum after removal of the cortex and the secondary folia is first presented in medial and oblique lateral views.

Incidentally, this is a useful method for visualizing the lobules and relation of vermis to lateral lobe folia. Then, representing the crest of the principal folia as segments of hoops, the course and relations of sample fibers are depicted stereographically, at first in their full extent, then cut at various levels to show other features.

Arcuate fibers are in 4 groups: A, a sagittal group connecting folia in the medial part of the vermis and extending a short way laterally in the superior semilunar lobule; B, a posterior group which forms an extensive connecting system between folia of the inferior semilunar, slender, and biventral lobules and tonsil; C, a system connecting lobules of pyramis and uvula in sagittal plane; D, connections between tonsil and posterior vermis.

Fibers from the vermis run forward in the anterior medullary velum. The medial ends of the semilunar lobules send fibers forward medial to the brachium conjunctivum and blending with it.

(See abstract of paper read by title for brachium pontis and restiform body.)

D 24. An appendix epiploica resembling a vermiform appendix. Homer B. LATIMER and Alfred H. HINSHAW, Department of Anatomy, University of Kansas.

This unusual appendix epiploica was first mistaken for the vermiform process by the medical students who were dissecting the cadaver. Later, a true vermiform process was found in the retrocaecal and retroperitoneal position.

In all of the texts on anatomy and on surgical anatomy consulted, no clinical significance was attached to these diverticulae. A search of the clinical journals showed that they are rather frequently the cause of sufficient abdominal pain to warrant operation. In none of the cases reported was the cause of the pain correctly diagnosed before operation.

This epiploic appendage was found in a 64-year-old, colored male who had died of heart disease. It was normal, not having undergone torsion which usually causes the necrosis of the contained fat. It was 3.5 cm in length and 7 mm in diameter. It was attached to the distal end of the caecum and lay parallel to, and slightly attached to the terminal part of the ilium. Because of its size, shape and location, it seems that during a laparotomy, if a similar condition should be found, the appendix epiploica might be mistaken for a true vermiform appendix.

Many cases of torsion of the epiploic appendages are reported in the literature and it does seem that a little more attention should be given to these epiploic appendages in the text books.

D 8. A new method for staining nerve fibers and endings using the Schiff reaction. H. M. LIANG, Department of Anatomy, Washington University. (Introduced by E. V. Cowdry.)

This is a simple and reliable method for staining nerve fibers and their endings of sensory, motor, cerebro-spinal or autonomic origin in whole mounts or sections. The staining principle is based on the assumption that certain chemical constituents of nerves (probably aldehyde in nature) will give a positive reaction with

Schiff's reagent. This premise was later sustained by actual observations. The procedure is a follows:

A. *Whole mount*

1. Fix tissue in 1% acetic acid or formic acid for 1 hr.
2. Wash in SO_2 water, 2 changes.
3. Transfer tissue into Schiff's leucobase for 1–3 hrs. or until the nerves are deep purple in color.
4. Wash thoroughly in several changes of SO_2 water.
5. Rinse with dist. water.
6. Counterstain in 1% methylgreen aq. sol. for 15 minutes.
7. Dehydrate, clear and mount.

B. *Section*

1–5. As in the above.

6. Embed, cut and remove paraffin as usual.
7. Counterstain in 1% methylgreen aq. sol. for 5 minutes.
8. Dehydrate rapidly through graded alc.
9. Clear and mount.

All glasswares and reagents should be Schiff negative.

D 44. Detached respiratory squames from the lung of the albino mouse. Charles C. MACKLIN, The University of Western Ontario Faculty of Medicine, London, Canada.

(See abstract of paper presented from platform.)

D 7. Certain aspects of peripheral ganglion cells in Fundulus. Samuel R. MAGRUDER, Department of Anatomy, Tufts College Medical School.

Histological preparations of peripheral ganglion cells of Fundulus prepared by the Nonidez method are presented. The ganglia studied are in the area of the anterior part of the stomach and liver, and are usually composed of large and small cells arranged in an irregular series along a nerve trunk. Many of the large cells contain complex polymorphic nuclei. Some nuclei are ring-shaped, some irregular elongate with folded nuclear membrane while some are five-lobed figures containing 8 or more nucleoli in addition to round droplet-like "bodies." Others contain 2 or 3 nuclei connected by thin strands of nuclear material. In addition there are a few cells which contain 3 to 5 nuclei between which no connection can be seen. These are always comparatively small and have never been seen to contain droplet-like bodies. General cell appearance, nuclear configuration and the presence of droplet-like bodies in the nucleus cause these cells to resemble those of the central nervous system described by Speidel ('22), Palay ('43) and others. Palay interpreted the characteristics as being evidence of nuclear secretion. However, only rarely has evidence been presented which indicated possible neurosecretory activity in peripheral ganglion cells (Lennette and Scharrer, '46). The preparations demonstrated here may possibly indicate neurosecretory processes although a complete secretory cycle has not been established.

D 5. Nerves of human dura mater. C. P. MARTIN, F. L. McNAUGHTON and C. L. LI, Montreal Neurological Institute.

The nerve supply of the dura mater was studied by anatomists in the nineteenth century, but little attention has been given to the dural nerves in recent years, though these nerves are undoubtedly of great importance in the understanding of head pain.

Full-thickness preparations of dura mater from middle and posterior fossae, tentorium, and falx, will be demonstrated, stained by silver nitrate and cleared in oil of wintergreen. Full details of the nerves in dura of the posterior fossa remain to be worked out. The remainder of the cranial dura receives its chief supply from the trigeminal nerve. The main dural branches can be followed grossly in these preparations, and compared with the diagrams of dural innervation.

D 12. Brain-modelling as a method for teaching advanced neuroanatomy. F. L. McNAUGHTON and John LORD, Montreal Neurological Institute. (Introduced by N. L. Hoerr.)

The brain modelling method of Adolf Meyer and Louis Hausman has been used at the Neurological Institute as the basis for the teaching of advanced neuroanatomy for Graduate Fellows, and as an elective course for upper year medical students interested in Neurology and Neurosurgery.

Reconstruction of the model must be based on the studies and observations of the student himself and this idea is stressed throughout. As source material, serial sections stained by Weigert-Pal, Nissl and Bodian methods are provided to supplement the student's own brain dissection, which includes fiber dissections of brain stem and cerebellar peduncles (Rasmussen). Tract degenerations demonstrated in human material, and experimental animal material are available for detailed study, and a bibliography of important articles introduces him to some of the certainties and uncertainties of neuroanatomy.

Lecture-demonstrations are arranged to fit in with the schedule of the modelling class, and in future will be combined with lecture-demonstrations in Neurophysiology by the Institute Physiologist.

By this pleasant method, the student provides himself with proven facts and a dynamic approach to the structure of the nervous system and, at the same time, prepares himself to proceed with original work.

The course occupies 2 evenings weekly for a 4-month period.

D 19. The embalming of fetuses and infants through the superior sagittal sinus. James A. MILLER, Department of Anatomy, Emory University School of Dentistry.

This technique for embalming fetuses, used for the past 3 years, is presented at this time because of the current nation-wide shortage of anatomical material and the possibility of using fetuses for certain types of dissections.

Studies by Tobler ('15) indicated that a needle inserted in the posterior angle of the frontal fontanelle to a depth of 3 to 6 mm will enter but not pass through the superior sagittal sinus. Batson's experiments ('40) showed that the verte-

bral veins provide a set of valveless venous channels connecting all parts of the body including the dural sinus system. Using the superior sagittal sinus as a portal of entrance of embalming fluid and the cut umbilical vessels for exit of blood, full term fetuses have been completely filled in less than 2 minutes at 5 lbs. pressure. Histological preparations from these fetuses indicate excellent penetration of fluid into all organs.

The only essential piece of equipment is an hypodermic needle of large caliber with an adjustable holder which prevents penetration to a depth greater than 5 mm, controls the angle of penetration and has a slot for an adjustable strap passing under the fetal chin. The demonstration will consist of the needle holder, photographs of fetuses from 4 months on embalmed with the technique, and histological preparations made from fetuses embalmed in this manner.

D 23. Electromyographic recordings from the m. biceps brachii during movements of the radioulnar, elbow, and shoulder joints. O. A. MORTENSEN, W. E. SULLIVAN and Meryl MILES, Department of Anatomy, University of Wisconsin.

The myograms were made with a 6-channel ink writing electromyograph having a paper speed of 3 cm a second. A pair of small surface electrodes was fixed to the skin (electrode past and celloidin) over each head of the muscle. Normal voluntary movements of the radioulnar, elbow, and shoulder joints were made by a subject in the sitting position, at average speed, with and without added load.

The electrical potentials were recorded when the biceps was acting to accelerate or decelerate a movement and when it was influencing motion as a topographical antagonist.

Supination of the radioulnar joint, flexion of the elbow joint, and flexion, abduction, adduction, and rotation at the shoulder joint represent acceleration.

Extension of the elbow, with gravity acting as the accelerating force, was used to illustrate deceleration.

Pronation of the forearm and active extension of the elbow were the movements used to illustrate the activity of the biceps when the muscle was acting as a topographical antagonist.

The records show variations in the amplitude of the potentials during the movements described. There are also variations which are the result of changes in the position of "accessory joints," load, and greater subjective effort.

D 20. Genetic growth differences as factors in the determination of bilateral symmetry in the rabbit. M. A. NACE and P. B. SAWIN, Department of Biology, Brown University.

The origins of the paired ilio-lumbar arteries in most races of rabbits, including races III and X, tend to be asymmetrical. The artery on the left side arises anterior to that on the right in approximately 60–70% of cases with 2–10% of the reversed type. Race V, in contrast, tends to have more symmetrical individuals and approximately 19% of the reversed asymmetrical type. The hybrid, F_2 and backcross generations between these races reveal the fact that the relative amount and direction of asymmetry appearing in these generations is correlated

with the relative mean antero-posterior level of the origins of these arteries. Thus the asymmetry appears to be determined by the relationship of the same growth forces, as have been shown in previous studies to influence the positions of the thoraco-lumbar and lumbo-sacral borders, and of the ventral spinous processes.

D 10. Sectional atlas of the brain of the adult albino rat. Wendell NICKELL and J. H. GRAVES, Institute of Neurology, Northwestern University Medical School. (Introduced by Wendell J. S. Krieg.)

A detailed cross-sectional atlas of the adult rat brain, consisting of 30 pairs of drawings, is presented. Alternate drawings from sections stained by the Weil method and with thionin were made. Additional illustrative material indicating the plane of section is included.

D 25. Developmental topographic anatomy of the infant thorax. Gustave J. NOBACK, Department of Anatomy, Cornell University Medical College.

Reconstruction by orthoscopic drawing of the pleural reflections, diaphragm, lungs, pericardium, heart and its valves, thymus, great vessels, etc., as projected against the thoracic cage and the vertebral column. The ages of the specimens utilized are, newborn, 10 days, 2 months, 3 months, 8 months, 11 months, 1 year, 1 year and 1 week, 15 months, and 2 years.

D 2. Changes in fiber tracts associated with inclusion bodies in nerve cells. James W. PAPEZ, Zoology Department Cornell University.

Inclusion bodies in nerve cells provoke changes in axon and sheath cells (oligodenendroglia). Sheath cells produce mucoid droplets found crowded between fibers of fiber tracts and at terminals of tracts.

Serum exudates from small blood vessels appear to be allergic reactions toward the mucoid bodies or other material. Patches of exudate may be widely disseminated in the c.n.s., but when in fiber tracts they lead to sclerosis. These exudates of serum (globulin), globular or racemose in shape, move and surround neighboring mucoid bodies, and form large laminated bodies. When a patch of such exudate spreads through tract of nerve fibers it blocks lymph spaces. Axons, myelin and sheath cells are engulfed and lymph supply is blocked.

Patches of exudate produce sclerosis: (1) myelin is removed by scavenger cells, (2) axons break up and disappear, and (3) sheath cells (oligodendroglia) give rise to dense fibers — the final sclerotic patch. Occlusion of small blood vessels is often seen in or near the patch, and sometimes an organized thrombus. Primary demyelination is another feature of affected tracts but depends to a larger measure on other factors or patches in some other region of the tract.

For inclusion bodies in nerve cells see abstract in Anatomical Record, suppl., *88:* 34, April, 1944.

D 6. Crossed fibers in the facial nerve in human embryos and fetuses. Anthony A. PEARSON, Department of Anatomy, University of Oregon Medical School.

(See abstract of paper from platform.)

D 39. Autographic studies of the bony tissues with radioactive phosphorus and of the thyroid with radioactive iodine. W. PERCIVAL, D. FINDLAY, J. GROSS and C. P. LEBLOND, Department of Anatomy, McGill University.

The distribution of radioactive phosphorus in the bony tissues of newborn animals was examined by several autographic techniques and was found to extend to all bones, ossification centers and tooth germs. This distribution was found to be completed as early as 1 hour after injection and to coincide with the distribution of alkaline phosphatase and of von Kossa's reaction.

The distribution of radioactive iodine was examined in the thyroids of animals in different experimental conditions (variable dietary iodine, stimulation with thyrotropic hormone, etc.:). In the active glands, the passage of iodine through the cells into an organic combination in the colloid is so rapid that, at no time after injection, is there enough iodine in the cells to make it visible by the autographic method. In resting glands, iodine occasionally may be found in the cells, especially soon after injection.

D 13. A brain stem model for teaching purposes. G. L. RASMUSSEN and S. PIAZZA, Department of Anatomy, University of Buffalo.

This model is intended to help the student visualize the continuity and the topographical relationships of the principal nervous pathways — not only in respect to neighboring structures of certain levels but also to the exterior of the brain stem itself. The various levels are of plexiglass on which are outlined the fasciculi, tracts, nuclei, etc. and they fit within a large half shell model of the brain stem. Individual neurons are fashioned to simulate their essential structure and to permit easy synaptic hook-up and rearrangement in order to demonstrate any particular functional system under study.

D 26. Spiral arteries in the ovary: injection-corrosion preparations. S. R. M. REYNOLDS, Department of Embryology, Carnegie Institution of Washington.

The arterial blood supply of the ovary in the rabbit is derived from 1 or 2 spiral arteries lying along the hilus of the ovary. The spiral arteries stem from the ovarian artery at the caudal pole of the ovary. The result of this arrangement is speculative, but hemodynamic principles suggest that it may serve to reduce arterial blood pressure to approximately osmotic levels and to equalize it throughout the ovary. This effect would be accomplished by decrease in the diameter of the vessel, increased length of vessel to be traversed by blood, and by change in the direction of blood flow (cf. Am. J. Obst. Gynecol., in press, 1947).

Injection-corrosion preparations in which vinylite was used as the injection mass will be demonstrated. A number of specimens will be shown in which there are casts of blood vessels to, and within corpora lutea. The effect of rapid follicular growth, ovulation and early luteinization will be demonstrated. In the early stages, the coils are "paid out" either by becoming less tightly coiled, or by becoming undulating in character. By the sixth to the ninth day after injection of gonadotrophins, the original configuration is laregly restored provided there are no large follicular cysts or corpora hemorrhagica. Photographs, stereoscopic transparent pictures in color, stereoscopic photographs, and original specimens will be demonstrated.

D 15. On a human ovum of 17 days development. G. Gordon ROBERTSON, Department of Anatomy, Baylor University College of Medicine.

An implanted ovum was recovered from an amputated uterus on the thirty-third day after the beginning of the preceding menstrual period. A block of tissue which included the chorionic vesicle was fixed in 10% formalin, embedded in paraffin, and sectioned serially at 6 μ. The plane of sectioning was found to be 10° from the transverse plane of the embryonic disc. A model of the embryo was constructed.

Comparison of measurements and morphological details with those of other early human ova indicates that the age is about 17 days. The external dimensions of the chorionic vesicle are 3.81 × 3.63 × 2.68 mm. The villi are branched, and the longest villus is 0.51 mm. Peripherally, the intervillous spaces have broad connections with venous sinusoids but no demonstrable connections with the spiral arterioles.

The embryonic disc is trilaminar. The ectodermal plate measures 0.46 × 0.48 × 0.04 mm. A primitive node and streak, a head process, and a cloacal membrane are present in the disc. The amniotic cavity has a volume of 0.0086 mm^3, the yolk-sac cavity a volume of 0.0209 mm^3. A small allantoic diverticulum extends from the yolk sac into the body stalk. Angiogenesis is evident in the chorionic and body-stalk mesoblast. Both angiogenesis and hemopoiesis are evident in prominent blood islands of the yolk-sac wall.

D 48. Isolation of the A- and B-cells in the pancreas of newborn puppies by the method of early duct ligation. Maria A. SERGEYEVA, Institut d'Anatomie Pathologique, Université de Montréal, and Department of Anatomy, McGill University. (Introduced by L. F. Bélanger.)

Partial ligature of the pancreas was carried out in a number of newborn puppies. The technique consisted of ligating the duodenal and splenic regions of the pancreas without affecting the blood supply of these ligated areas. The animals were sacrificed from 2 to 16 weeks later.

In the ligated duodenal end, the normal acinar structure was at first transformed into coiling strands of oval or rounded cells with prominent, voluminous nuclei. Later on, an abundant proliferation of B-cells arose from these strands, while they themselves gradually became thinner. Maximum B-cell prolif-

eration occurred between the eighth and twelfth week and was then followed by atrophy of the ligated segment.

In the ligated splenic end, the acini underwent a similar transformation. These, however, showed an A-cell proliferation which was maximal at 8 to 12 weeks after ligation.

This difference may be correlated with differences in innervation and embryological origin of these 2 different regions.

This operation reveals the potential ability of acinar cells to become transformed into endocrine cells.

D 49. The origin of fat cells in the involuting thymus. Christianna SMITH, Department of Zoology, Mount Holyoke College.

In age involution, the thymus of the mouse and the rat becomes replaced in varying degrees by fat cells. For the most part, the intrathymic, subcapsular fat cells develop in situ from lymphoid cells by the gradual accumulation of fat droplets which fuse finally to form 1 large globule in them.

D 3. The "acid" phosphatase content of normal autonomic neurons and its increase following section of the cell processes.[1] Wilbur K. SMITH and Charles LUTTRELL, Department of Anatomy, The University of Rochester, School of Medicine and Dentistry.

The phosphatase content of autonomic neurons was investigated by a modification of the histochemical method of Gomori ('41), the most important alterations being the fixation of the tissues by perfusion with 20% formalin in Ringer's solution and incubation of the sections in the glycerophosphate substrate at pH 4.7–5.0 for a longer time.

The neurons in normal stellate ganglia prepared in this manner show comparatively little phosphatase reaction, the cytoplasm being only lightly stained. A narrow zone around the nucleus shows a greater response. The nucleolus stands out darkly stained among smaller, scattered and more lightly appearing nuclear granules. Nerve cell processes react to approximately the same degree as the cytoplasm. The majority of the nuclei of the peri-neuronal cells appear to have a phosphatase content greater than that of any part of the neuron itself, while the nuclei of the cells of connective tissue enclosing the ganglion show less enzyme reaction.

Chromatolysis in neurons following section of a branch of the stellate ganglion is accompanied by a great increase in phosphatase content of the cytoplasm. In the central nervous system, the cells of the dorsal motor nucleus of the vagus show increased phosphatase following section of the vagus nerve.

[1] Aided by a grant from The National Foundation for Infantile Paralysis.

D 1. Osmophilic granules in chromatolytic nerve cells. W. A. STOTLER, Department of Anatomy, University of Oregon Medical School. (Introduced by O. Larsell.)

Distinct granules blackened by osmic acid are seen in chromatolytic cells whose axons have been severed in sections of the rat brain stained by the Swank-Daven-

port method and counterstained with neutral red-toluidene blue. The granules appear early in the chromatolytic process and form a critical method of identifying affected cells. Granules are demonstrated in the red nucleus following lesion of the rubro-spinal tract, in the ventral tegmental nucleus following lesion of the mammillary peduncle, and in the nuclei of cranial nerves following interruption of their axons. These granules constitute a possible source of error in identifying degenerated myelinated pathways.

D 14. Vascular patterns of development in the primitive neural tube of the rabbit. Leon H. STRONG, Department of Anatomy, University of Michigan.

(See author's abstracts under paper from platform and paper read by title.)

D 41. A comparison of smooth muscle myofibrillae in golden hamster, albino rat and rhesus monkey. Donald C. TANNER, Department of Anatomy, Stanford University. (Introduced by Hadley Kirkman.)

Slides of Bouin fixed muscle, stained by Bodian's protargol method, illustrate the great relative superiority of hamster material for the preparation of exceptionally beautiful demonstrations of mammalian smooth muscle myofibrillae.

D 46. A histological transformation of intestinal mucosa following administration of alloxan. Harry C. TRIMBLE and Harold L. WEATHERFORD, Departments of Biological Chemistry, and of Anatomy, Harvard Medical School.

Shaw Dunn and his associates observed that the primary effect after the administration of alloxan to animals was a selective necrosis of the islets of the pancreas, and the animals became diabetic. No significant changes were seen in other organs.

We have given alloxan to dogs over varying periods and find that when included in the food, there was a temporary elevation of blood sugar and no sugar in the urine. But when alloxan in solution is injected intravenously a severe diabetes ensues. Besides degenerative changes in the islets of Langerhans, we find a remarkable transformation of the epithelium, from columnar to flat, covering the villi of the small intestine. The epithelium of the intestinal glands seems less affected, there being no reduction in cell heights, but from the crowded appearances near the base of the glands there are observed evidences of cellular increase. The villi as a whole look swollen, and the bundles of smooth muscle fibers in them hypertrophic.

D 50. Accessory thyroid tissue and thymus IV in baboon. John H. VAN DYKE, Department of Anatomy, Washington University School of Medicine.

An *atrophic* thymus IV, infiltrated with eosinophiles and associated with developing thyroid tissue within the same connective tissue capsule, has been identified in an adult male baboon (Papio anubis; Subgenus Choerapithecus).

A large medullary cyst, possessing simple columnar epithelium (basophilic and serous secretions) and, in part, *hyperplastic* stratified squamous epithelium with pearl formations, intervened between thymus and accessory thyroid tissue. Cyst extensions, continuous with small (young), densely staining thyroid-like vesicles, were contiguous with large vesicles (older) undergoing transformation into a typical accessory thyroid lobe, the caudal end of which was attached by a strand of follicles to the main thyroid mass at the site of entry of parathyroid IV and ultimobranchial tissue. Although rich in colloid and colloid cells, both thyroid bodies appeared *active;* typical chief cells were rare. Clear cells, however, with large brown cytoplasmic granules, following potassium dichromate fixation, were conspicuous. Globular secretions (serous; frequently basophilic), derived only from such cells, mingled with acidophilic colloid.

The evidence has been interpreted as indicating that thymic cytoreticulum or ultimobranchial tissue left outside the thyroid gland during embryonic development (occasionally as cysts) may be induced to form accessory thyroid tissue *after birth,* as in sheep (Van Dyke, '45), and that resorption of colloid, apparently secreted by *colloid cells,* is facilitated by a proteolytic enzyme that may be derived from these granular cells.

D 16. A study of closure of the pleuropericardial and pleuroperitoneal canals in the human embryo. L. J. WELLS, Department of Embryology, Carnegie Institution of Washington and Department of Anatomy, University of Minnesota.[1]

In examining serial sections of 221 embryos of the Carnegie Collection (25 somite to 27 mm stages, Streeter's Horizons XII to XXII), it was found that both pleuropericardial canals are closed in 32 embryos of Horizon XVIII (12 to 17.2 mm). Both pleuroperitoneal canals are closed in 23 embryos of Horizon XX (16 to 25 mm).

To assist in studying the manner of closure, 3-dimension reconstructions of 4 embryos were prepared from sheets of cellulose acetate. These models will be demonstrated (4.0, 8.3, 9.2 and 14.5 mm embryos).

Pleuropericardial canals disappear when common cardinal veins fuse with primitive mediastinum. This process is completed earlier on left side than on right. Thereafter those paired membranes that separate pleural and pericardial parts of coelom and that contain phrenic nerves, resemble "mesenteries" of right and left portions of sinus venosus. These membranes contain mesenchyme of origin of part of that endothoracic fascia which loosely binds pleural sacs to pericardial sac of adult.

It would seem that pleuroperitoneal membranes, initially ridges of mesothelium plus mesenchyme they contain, owe their origin to fact that lung buds are too large to pass through very top of paired archways between posterior cardinal veins and sinus venosus. Narrowing of pleuroperitoneal canals involves at first the rapid growth of liver and Wolffian bodies (on left side, stomach as well). Closure occurs soon after suprarenals contact liver.

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[1] Fellow of the John Simon Guggenheim Memorial Foundation.

D 45. Effect of alloxan on the pancreatic islets of the guinea pig demonstrated with janus green. Charles A. WOERNER, Department of Anatomy, Long Island College of Medicine.

Twenty guinea pigs were given alloxan in doses of 50 to 200 mg per kilo, intravenously. Twenty-four hours after the alloxan was given the animals were perfused with janus green using the method described by Bensley. Pieces of pancreatic tissue taken from the head, body, and tail of the pancreas of each animal were then washed in water, dehydrated in alcohol, cleared in benzol, and mounted in balsam. For comparison 8 apparently normal guinea pigs were perfused with janus green and the pancreatic tissue prepared in the same manner.

The larger islets appear to show more degeneration than the smaller islets in that many of the cells of some of the larger islets fail to stain with the janus green. While there appears to be some decrease in the number of large islets, the number of small islets does not appear to be greatly reduced.

MOTION PICTURE DEMONSTRATIONS

Dm 59. A new method of semen analysis.[1] Edmond J. FARRIS, The Wistar Institute of Anatomy and Biology.

A method of semen analysis based on counting the number of moving cells is shown. The new procedure correlates the factors of volume, count per cm^3, and actual percentage of motility, to give the absolute motility, which is the actual number of moving spermatozoa in the ejaculate.

The findings obtained from 200 semen analyses on over 150 individuals has resulted in an index of fertility.

Since fertility is dependent upon moving sperm, it is felt that a correlation of the many criteria rather than individual factors is the more reasonable explanation of fertility.

Males are classified as high fertile, relatively fertile, or infertile, as determined by the absolute motility.

Photomicrographs were made by phase microscopy.

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[1] This investigation was aided by grants from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry of the American Medical Association, and the Samuel S. Fels Fund.

Dm 58. Serial levels through an embedded specimen recorded by direct photography of the block surface. Erling S. HEGRE and Alton D. BRASHEAR, Department of Anatomy, Medical College of Virginia.

Embryos prepared for block-surface staining (see Anat. Rec., *97:* 21–28) were mounted vertically in a sliding-knife microtome. A 16-mm movie camera supported above the specimen was used in recording the details of the embryo as seen on the cut surface of the block at successive levels. The completed film is suitable for continuous projection or it may be used in making reconstructions of the specimen.

Dm 53. A colored moving picture used in teaching gross anatomy. W. Henry HOLLINSHEAD and J. E. MARKEE, Department of Anatomy, Duke University School of Medicine.

The reel to be shown is an excerpt from approximately 15,000 feet of colored motion pictures prepared in our department as an aid in teaching gross anatomy. These pictures show demonstration dissections of most of the regions of the body. During the dissection, nerves and blood vessels were painted, so that they might show up more clearly. Then, under the movie camera, the important structures in a given region were pointed out, moved about to show their relations, and, when necessary, removed to display deeper structures.

This film has been used by us in teaching gross anatomy to nurses, physiotherapists and first year medical students, and also in presenting reviews of anatomy to both undergraduate and graduate students. The level of instruction, and the structural details stressed, can be varied within wide limits by supplementation with appropriate lantern slides.

Dm 57. Hypokinesia and the retention of superimposed posture in the primate as a result of neural lesion. Fred A. METTLER, Department of Neurology, College of Physicians and Surgeons, Columbia University.

Overactivity as a result of cortical removal and essential overactivity with fatuity, as a result of striatal ablation have been described by Hines and Richter, Mettler and most recently by Heath and Freedman. The motion picture to be presented illustrates the effect of bilaterally removing all cortex rostral to the middle of area 6, the heads of the caudate and most of the pallida.

Addition of pallidal removal converts overactivity into underactivity. There is a chronic loss of adaptive activity to disturbing situations, stereotypy of movement and apparent drowsiness. Flexor plantar responses may be obtained, the patellar reflexes are brisk, easily elicited and show good amplitude. In addition, superimposed postures are maintained. Such a condition does not depend upon hypothalamic damage.

The current interpretation of this condition suggests that cortical and striatal removal release thalamopallidal circuits dealing with stereotyped varieties of movement (associated movements) which depend to a large extent upon proprioceptive stimulation and that this circuit is interrupted by pallidal removal. Such an interpretation agrees with the observed loss of associated movements which is encountered in various clinical conditions such as lenticular degeneration.

Dm 55. Cinephotomicrograph of pure thymus epithelium.[1] Raymond G. MURRAY, Department of Anatomy, Tufts College Medical School. (Introduced by Benjamin Spector.)

This film combines representative portions of many cinephotomicrographs of epithelium from cultures of rabbit thymus ranging in age from 1 day to 1 year. Typical epithelial growths in the form of "tongues" projecting from thymus cultures are shown, as well as the subsequent activity of the pure epithelium resulting from cutting away the rest of the culture. After several days in vitro

this pure epithelium is shown exhibiting pinocytosis and possibly phagocytosis, and at times growing in a manner resembling connective tissue. A more typical mode of growth, however, is that of anastomosing cords of flat cells. Two instances of what appear to be differentiating mitoses are shown. In each case 1 daughter cell returns to the epithelial cord and the other moves about in a manner somewhat suggestive of a lymphocyte, although much slower. Such mitoses occurred only twice, and both times while the culture was under low oxygen tension. Gall bladder epithelium, cultured and photographed under similar conditions is included for comparison.

[1] Aided by a grant from the Abbott Memorial Fund of the University of Chicago.

Dm 54. Dynamics of normal and pathological temporomandibular articulations. William M. ROGERS and S. L. KATZ, Departments of Anatomy and of Dental and Oral Surgery, College of Physicians and Surgeons, Columbia University.

These pictures show the types and limitations of movement in a variety of disorders of the temporomandibular joint. Results following surgical interference in selected cases, are presented.

Laminagrams of normal and malfunctioning articulations are used as an aid in studying their anatomy and their function.

Dm 52. Motion of the ankle and foot produced by individual muscles in the leg. Charles E. TOBIN and Francis F. BAKER, Department of Anatomy and Division of Orthopedics (Department of Surgery), The University of Rochester, School of Medicine and Dentistry.

A dissected, unembalmed leg and foot were photographed on Kodachrome film to show the action of the muscles in the leg on the movements of the tarsus, metatarsus and digits.

The muscles were isolated, each identified by a label, and its contraction simulated by mechanical tension.

This film is offered as a technique to aid in teaching the action of these muscles in their functions as extrinsic muscles of the foot.

Dm 56. Circulatory flow pattern in the bat's wing. Richard L. WEBB and Paul A. NICOLL, Departments of Anatomy and Physiology, Indiana University Medical School.

Observation of peripheral blood circulation in the subcutaneous tissue of the wing membrane of the living bat is possible by use of the compound microscope. Records of vascular pattern and behavior are recorded by cinematography for this demonstration. The arrangement of the vascular system to the capillary bed is in the form of a series of arcades. The capillary system proper is a complex net of endothelial tubes, supplied by terminal arterioles and drained by coalescing venules connecting with the arcuate vessels. No preferential flow paths or arteriovenous anastomosis have been observed.

Active vasomotion on the arterial side of the system is more pronounced as the capillary bed is approached, being manifested mostly in the terminal arterioles and precapillary sphincters, where it is rhythmic in nature. On the venous side of the circulation, active vaso-motion is resumed at the location of the first venous valve. From here on the rhythmic movement is definite, apparently concerned with the propulsion of blood in its return to the heart.

By use of methylene blue vital stain, some of the muscular elements are shown. Of especial interest is the coiled or spiral muscle cells of the terminal arteriole. Arrangement of muscle fibers in the region of venous valves and junctions is shown.

INTERPHASE (RESTING) NUCLEI, CHROMOSOMAL VESICLES AND AMITOSIS[1]

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EIGHT FIGURES

INTRODUCTION

Interphase nuclei including those of normal and malignant fibroblasts probably consist of closely packed, firmly adherent, swollen chromosomes (chromosomal vesicles or karyomeres). This concept is based on the following considerations. (a) In early cleavages of invertebrate, fish and amphibian eggs chromosomes remain separate at end of anaphase, swell into discrete chromosomal vesicles during telophase and then become adherent into compact interphase nuclei. (b) Aberrant chromosomal vesicles are found in the cytoplasm after cells are subjected during mitosis to certain agents and in some malignant cells in ordinary hanging drop cultures. (c) Loss of adhesion between chromosomal vesicles offers an explanation for the mechanics of amitosis, partial cleavage and fragmentation of interphase nuclei without cytoplasmic cleavage. (d) It helps to explain certain nuclear phenomena of normal and malignant fibroblasts and other cells, namely, (1) shrinkage of individual chromosomes during prophase and swelling of the daughter chromosome mass during telophase; (2) The maintenance of nucleolar patterns (number, location, size, shape) during interphase because adhesion of chromosomal vesicles would prevent nucleolar shifting; and (3) The mainte-

[1] Aided by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council and by a former grant from the International Cancer Research Foundation. Continuation of studies begun at the Department of Embryology, Carnegie Institution of Washington.

nance of chromosome continuity during interphase. A clear understanding of interphase nuclei is essential for understanding mitosis.

NORMAL OCCURRENCE OF SEPARATE KARYOMERES

It has been known for many years that during maturation and early cleavage of many invertebrate, fish and amphibian eggs the chromosomes remain separated from one another at the end of anaphase and during telophase when they swell into chromosomal vesicles. They become adherent to each other during interphase to form a compact nucleus. In some forms the vesicles can be recognized during interphase.

Conklin ('02), p. 45 states, "Such vesicles are found generally, if not universally, in early divisions of ova, though they are not usually found in other mitoses;" and p. 47, "It is altogether probable that the chromosomes and hence the chromosomal vesicles, preserve their identity through the resting period, and I venture to suggest that the daughter chromosomes will be found to arise within the chromosomal vesicles."

Richards ('17) observed chromosomal vesicles in Fundulus. He gives a long list of authors who recognized them previous to 1917. To this should be added Schwarz (1888) fish, cleavage; Braus (1895) triton, cleavage; Conklin ('02) Crepidula, cleavage. Since then numerous observers have noted vesicles in invertebrates, fishes, amphibia and plants.

The widespread occurrence of the swelling of chromosomes into separate vesicles during telophase and their subsequent adhesion to form interphase nuclei leads one to suspect that all chromosomes swell during telophase and that all interphase nuclei consist of adherent chromosomal vesicles or karyomeres.

The absence of separate vesicles in mitosis of many adult cells and in birds and mammals can be attributed to the adhesion of chromosomes at the end of anaphase or beginning telophase before they begin to swell instead of at the end of telophase after they have swollen into vesicles.

ABERRANT CHROMOSOMAL VESICLES

Aberrant chromosomal vesicles come from lagging chromosomes that fail to reach the poles during anaphase and so fail to become incorporated in daughter nuclei. They get into the cytoplasm after the spindle gel has retracted to the poles and after the spindle sap in which the the aberrant chromosomes lie becomes mixed with cytoplasm. This mixing usually occurs just after cytoplasmic cleavage. Vesicles differ in size, some are single others multiple.

Aberrant chromosomes sometimes occur in certain types of malignant cells cultivated in ordinary hanging drops without the addition of any special agents (Lewis and Lewis, '32). Schwarz ('46) has noted them in abnormal blood cells taken directly from bone marrow.

They have not been noted in normal cells. They can be produced by the treatment of cells with different agents, Conklin ('12) hypertonic sea water, mollusks; Conklin ('17) high temperature, mollusks; Whitman ('33) radium, chick embryo fibroblasts in cultures; Rosenfeld ('33) ammonia, chick embryo fibroblasts in cultures; Politzer ('34) röntgen irradiation, cornea of Urodele larvae; M. R. Lewis ('35) fluorescent X, chick embryo fibroblasts in cultures; Nebel and Ruttle ('38) colchicine, stamen hairs; Beams and Evans ('40) colchicine, Arbacia punctulata eggs first division; Hughes-Schrader and Ris ('41) irradiation, coccids; Steinitz ('44) lack of oxygen, tradescantia, meiosis.

The factors concerned in the production of aberrant chromosomes are probably, (1) failure or too long delay of separation of 1 or more chromosomes into the 2 daughter ones at the end of metaphase, (2) failure of the spindle or local parts of it to pull 1 or more daughter chromosomes to the poles. In either case they lag behind and are not incorporated in daughter nuclei.

Aberrant chromosomes swell into vesicles presumably simultaneously with the swelling of those that compose the daughter nucleus. When the chromosomes of the latter again shrink for the next mitosis there is a simultaneous shrinkage

of the aberrant vesicles into dense chromosomes (Lewis and Lewis, '32, figures 118–119; Politzer, '34; Clark, '40; Hughes-Schrader and Ris, '41).

The fact that aberrant chromosomes swell into vesicles and that they give rise to dense chromosomes simultaneously with the appearance of chromosomes in the adjacent nucleus as it goes into mitosis suggests that the interphase nucleus also consists of adherent chromosomal vesicles.

AMITOSIS

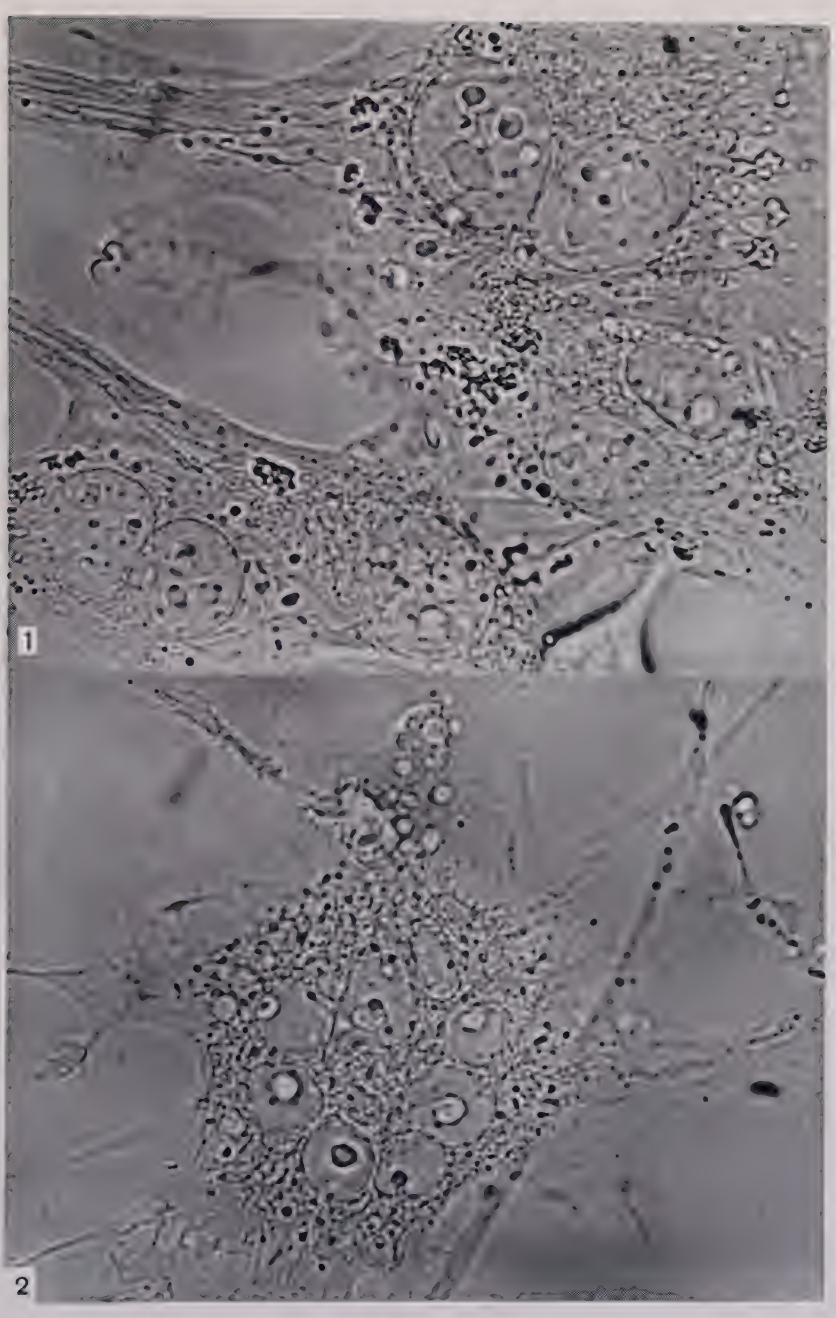
There are many indications (binucleate cells, cleft nuclei and fragmentation of the nucleus) that amitotic division of the nucleus without division of the cytoplasm is common in ordinary hanging drop cultures of both normal and malignant fibroblasts. My observations agree in general with those of Macklin ('16) who maintains that binucleate cells are normal and are due to amitotic division of the nucleus.

Almost every culture contains binucleates and some have many. Figure 1 shows a mononucleate, a binucleate and 2 cells each with a large nucleus nearly cleft into 2 parts. All 4 cells seem to be in good condition with long mitochondria and a moderate number of fat globules. There is no particular difference in the cytological characters of the 4 cells. There are no mitotic changes. The binuclear and cleft nuclear conditions are clearly amitotic in origin.

Photographs × 1100 dia. of living cells in hanging drop cultures. Medium for figures 4, 5, 6, 7, 8, consists of 7 parts Gey's saline plus 2 parts human cord serum plus 2 parts chicken plasma plus 2 parts embryo juice.

Fig. 1 Malignant fibroblasts, 1 uninucleate, 1 binucleate with completely separated nuclei, 2 cells each with nucleus almost but not completely split into 2 parts, cells in good condition, many mitochondria, moderate amount of fat. Mouse sarcoma BA2, 10 generations in vivo plus 120 days in roller tubes plus 82 generations in vivo, 4 day hanging drop culture, 1 part chicken plasma plus 1 part beef embryo juice.

Fig. 2 Large malignant fibroblast, same culture, 8 small nuclei probably derived by repeated splitting of a binucleate, cell seems to be in good condition, numerous mitochondria, active pseudopodia and some pinocytosis.



Figures 1 and 2

All degrees of cleft nuclei are encountered from ones with a small cleft or indentation to those that are almost divided (figs. 1, 3, 4). The clefts may increase and decrease in depth and some of them probably never proceed to complete separation of the nucleus into 2 parts. The small opening at the bottom of the cleft in figure 3 suggests that the loss of adhesion is complete here and not quite complete along the rest of the cleavage line. Such a condition can be explained by loss or partial loss of adhesion between vesicles better than by a constriction band. The 2 parts are sometimes widely separated except for a connecting strand as in figure 4. Sometimes the 2 parts are closely pressed together and more or less flattened against each other and it may be impossible to determine if division is complete.

Although such observations indicate that nuclear amitosis is common, Macklin reports following the process in only 1 case. It took about 1 hour. He found as I have that clefts may regress and the nucleus become smooth. I succeeded in the course of a few attempts in following 2 complete amitotic divisions of the nucleus and 2 polar fragmentation cleavages (figs. 5, 6).

There are many indications that each nucleus of a binucleate may divide and subdivide into smaller and smaller ones, pro-

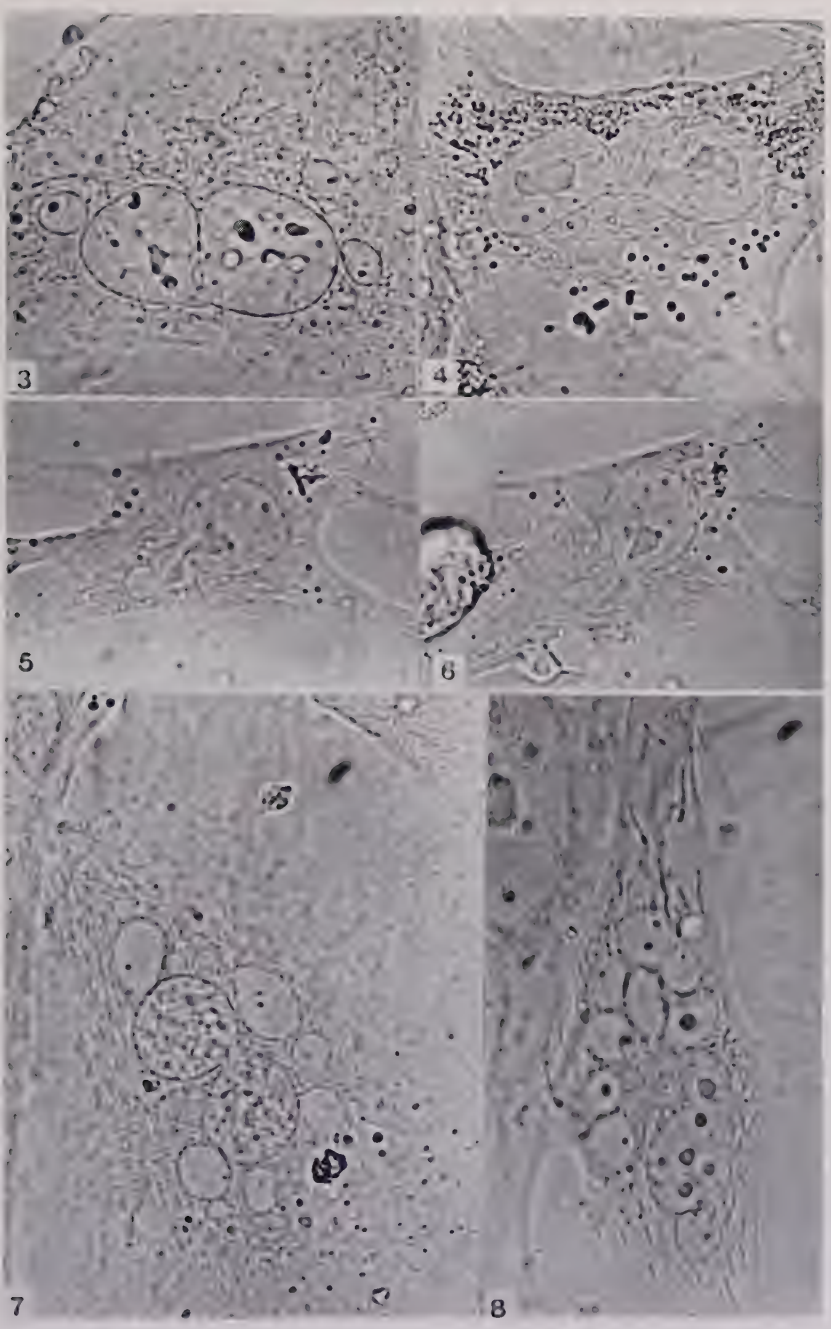
Fig. 3 Central part of a flat fibroblast, 1 day mouse, large nucleus nearly split in two, 3 small nuclear fragments near it, mitochondria abundant, 6 day culture, 4 saline plus 3 horse serum plus 2 embryo juice.

Fig. 4 Fibroblast adult rat, 2 nuclei connected by a slender strand, cell in good condition, 55 days in roller tube and 2 days in hanging drop culture.

Figs. 5 and 6 Fibroblast, adult rat. Figure 5 nuclear fragment nearly split off. Figure 6 40 minutes later, fragment seems to be completely separated. 4 day culture.

Fig. 7 Malignant cell of Crocker rat sarcoma no. 10, large nucleus nearly split in two, prophase-like appearance, 9 small fragments, 3 smallest ones are without nucleoli and may be individual chromosomal vesicles, cell is flattened on the coverglass, many mitochondria, few fat globules, 7 day culture.

Fig. 8 Fibroblast from 12 day old mouse, 10 nuclear fragments and another one partially split off from the main nucleus which is reduced in size, 189 days in roller tubes and 4 days in hanging drop culture.



Figures 3 to 8

ducing fragmented nuclei. Such cells are usually considerably larger than average but otherwise appear normal (fig. 2).

Fragmentation often begins at one or both poles of a more or less elongated nucleus (figs. 5, 6). Cells with 1 to many completely separated fragments are more common in older cultures than in fresh ones (figs. 3, 7, 8). Complete fragmentation into the total number of chromosomal vesicles has not been noted. It is a possibility.

Large nucleoli consist of an agglutination of several smaller ones each connected with its own chromosome. Nucleoli are occasionally split during cleavage (figs. 5, 6). This indicates loss of adhesion between chromosomal vesicles whose nucleoli had previously agglutinated into a common mass. Most fragments have 1 or 2 nucleoli (figs. 3, 7, 8). Fragments with 2 nucleoli probably consist of 2 or more chromosomal vesicles. Those with 1 small nucleolus may consist of a single vesicle or of a vesicle with a nucleolus and 1 or more without nucleoli. The 3 smallest fragments in figure 7 are probably single chromosomal vesicles without nucleoli. Each part, figure 3, has a large nucleolus. This suggests that the cleft is between 2 groups of chromosomal vesicles, each group being connected with a central compound nucleolus.

Mechanics of amitosis. If the interphase nucleus consists of closely packed adherent chromosomal vesicles and if its membrane is an adherent mosaic of their outermost walls that are at the surface, one can explain amitosis, partial cleavage and fragmentation, as due to the loss of adhesion between groups of chromosomal vesicles rather than to the constriction of a band of the nuclear membrane as Macklin has suggested. Loss of adhesion would automatically be followed by a rounding up of each part. When the 2 parts are close together as in figure 3, the loss of adhesion is probably somewhat incomplete along the cleft line.

The factors responsible for loss of adhesion between groups of chromosomes are unknown. It is presumably concerned with metabolism.

CHROMOSOME BEHAVIOR DURING MITOSIS OF LIVING CELLS

The concept that interphase nuclei of adult normal and malignant fibroblasts consist of chromosomal vesicles offers a key for partial explanation of certain mitotic phenomena.

During prophase chromosomes become visible as short rods, presumably firm gels, that appear to be separated from one another by clear fluid. This can be ascribed to shrinkage of the chromosome of each vesicle and to the separation out of fluid. During this process chromosomes lose their adhesiveness for one another because of the fluid that has accumulated between them. The nuclear membrane, which according to Conklin ('02) Crepidula; Weinrich ('16) Phrynottetix; Richards ('17) Fundulus; Swingle ('21) Anurans; Kater ('26, '27, '28) plants, frogs, rats; Eisentraut ('26) Dedipoda; Smith ('29) Cryptobranchus; Bragg ('38) Bufo; Hughes-Schrader and Ris ('41) Coccids, and others probably consists of an adherent mosaic of the outermost walls of the chromosomal vesicles, now loses its continuity. The chromosomes then become free from one another and are pulled into the metaphase plate by the spindle gel which had previously become attached to them.

At the end of anaphase chromosomes come into close contact with one another and form a firmly adherent mass. During telophase the adherent mass swells, and the chromosomes become invisible. Presumably each chromosome swells into a vesicle but their firm adhesion prevents their recognition. At the end of telophase (chromosome swelling), the interphase nucleus stage begins.

DISCUSSION

It is obvious that one cannot understand mitosis without a clear understanding of the interphase nucleus. The concept that it consists of adherent chromosomal vesicles harmonizes with what one finds in prophase and telophase.

The factors responsible for adhesion and swelling of chromosomes during telophase, maintenance of this condition during interphase, shrinkage during prophase and the mainte-

nance of this dense gel state during metaphase and anaphase are unknown.

Our knowledge of chromosome structure is so uncertain that one can only guess at what happens during shrinkage and swelling. Schrader ('44) presents a diagram modified from Geitler ('38) in which the following parts are depicted, genonema (or chromonema), matrix, pellicle (or sheath) and kinetochore (or centromere). Probably both genonema and matrix are gels that have the property of swelling and shrinking. The sheath may be a more viscous superficial layer of the matrix. These sheaths may correspond to the linin sheaths of the chromosomal vesicles, Conklin ('02), which according to Kater ('27, '28) persist in the resting stage of frogs and rats and can be seen as linin strands in fixed and stained nuclei.

The compartmentalization of the nucleus by adhesion of its chromosomal vesicles helps to explain how nucleolar pattern (number, location, size, shape) are maintained during interphase, Lewis ('40). Most nucleoli consist of several adherent small ones, each is connected with its chromosome and since the latter are also adherent shifting tends to be prevented. Nucleoli may change in shape and size but not in number and location during interphase.

The maintenance of chromosome continuity and individuality during interphase would seem to require that each swollen chromosome retain its individuality (Conklin, '02; Wenrich, '16; Richards, '17 and others). Adhesion, not fusion into a common nuclear vesicle, would provide the conditions for this.

Cells spend most of their lives in interphase. Nuclear metabolism is dependent upon passage of fluids in and out of chromosomes and this can probably be carried on more effectively when they are swollen into thin gels than when they are in the dense mitotic state. In the latter state they can be more readily moved by the spindle apparatus.

Macklin ('16) states on page 100 that, "The paired nuclei of binucleate cells in tissue cultures arise by direct division of the nucleus, or nuclear amitosis, without division of the

cytoplasm. This occurs in perfectly normal cells''; and on page 101, ''Nuclear fragmentation was found to occur where conditions for life were not favorable, and was thus a form of degenerative change.'' One suspects however that even cleavage into 2 normal appearing nuclei in what appears to be a normal cell is the result of some invisible change in the nucleus which is not quite normal. Both amitosis and mitosis seem to follow an increase in nuclear size but the factors that determine which will follow are unknown. There are indications of increase of both nuclear and cytoplasmic mass after amitosis and fragmentation. There are, however, no growth curves available for either normal or amitotic cells during the interphase period.

The possibility that some binucleate cells may arise by mitotic division of the nucleus without division of the cytoplasm or by fusion of cells should be considered since both occur in cultures but they seem to be too rare to account for all binucleate fibroblasts. Mitosis does not seem to play any role in partial cleavage or nuclear fragmentation.

CONCLUSIONS

Interphase nuclei consist of firmly adherent swollen chromosomes (chromsomal vesicles or karyomeres).

Chromosomes of normal and malignant fibroblasts become adherent at the end of anaphase, then during telophase swell into vesicles that remain adherent to one another. Individual vesicles retain their identity throughout interphase. During prophase each chromosome shrinks into a dense gel which is separated from its neighbors by the fluid which collects between them.

Loss of adhesion between chromosomal vesicles of interphase nuclei is responsible for amitosis, partial cleavage and fragmentation of interphase nuclei without cytoplasmic cleavage.

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STUDIES ON LUNG TISSUE IN VITRO WITH SPECIAL REFERENCE TO THE NATURE OF THE LINING CELLS OF THE ALVEOLUS

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SEVENTEEN FIGURES

The present study on lung tissue cultivated outside the organism was undertaken in the hope of extending our knowledge regarding the nature of the lining cells of the pulmonary alveoli by investigation of their developmental potentialities in vitro.

METHODS

Material. Lungs from 8-week-old rabbits were used. The lungs were cut with fine scissors into pieces about 3 mm^3 in size. In some experimental series lung fragments taken from the edge of the pulmonary lobe and containing visceral pleura were explanted. In other series the explantation was carried out without visceral pleura, the fragments being excised from the cut surface of a pulmonary lobe.

Method of explantation. For the explantation of lung we used the method described elsewhere by Rachmilewitz and Rosin. Immediately after removal the lung fragments were placed into the medium consisting of 3 drops of heparinized rabbit plasma, 3 drops of Tyrode's solution and 1 drop of diluted chick embryo extract. The tissue fragments were planted at the time when the plasma began to coagulate, so that they remained suspended in the uppermost layers of the medium. The explantation was carried out in glass tubes,

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2.5 cm long and 8 mm in diameter, tightly closed with cork stoppers. The cultures were incubated at 37°C. for 24, 48, 72 and 120 hours respectively. After incubation the plasma clot containing the explant was removed and sectioned for histological examination. For the study of the peripheral outgrowth some of the fragments explanted in tubes were further cultivated through many passages as hanging drop cultures or using the Carrel flask method. Such cultures were examined in toto.

Histological technique. The cultures for in toto examination were fixed in Carnoy's fluid and stained with Giemsa's stain. The explants in tubes were fixed in Zenker's solution and embedded in celloidin-paraffin. Serial sections 4 μ in thickness were stained with hematoxylin-eosin and with Giemsa's stain.

FINDINGS

I. Observations on the outgrowth developing around the explanted lung fragment

a. Lung fragments explanted without visceral pleura. Seven to 9 days after explantation the lung explants cultivated in hanging drops or Carrel flasks regularly showed, in addition to scarce spindle cells, solid cell membranes consisting of polygonal cells in pavement arrangement around the explant. The edges of the individual cells forming the membrane were closely adherent to each other. As a rule the individual cells could be easily discerned, although in some places the intercellular boundaries were unrecognizable and the membrane had a syncytial aspect. The cytoplasm of the cells stained light blue with Giemsa's stain and was frequently vacuolated. The nuclei were spherical or oval in shape, poor in chromatin and contained 1 to 2 nucleoli. Mitoses were numerous (fig. 1, 2). The peripheral border of the membrane formed an undulating line. No ciliation of the cells composing these membranes was noted. In cultures 12 to 16 days old the cell membranes became more irregular in shape. They formed branching processes, which grew in tree-like fashion (fig. 3, 4).

The outgrowth observed around the lung fragments explanted without visceral pleura thus showed all the characteristics of epithelial membranes variously described in cultures of typical epithelial tissue.

b. Lung fragments with visceral pleura. The outgrowth from lung fragments explanted together with visceral pleura and cultured in hanging drops and Carrel flasks were morphologically quite different from the cultures described above. In hanging drop cultures 8 days after explantation extensive and uniform cellular membranes could regularly be seen around the explant beyond the outgrowth of scarce fibroblasts. These membranes consisted of a thin and very delicate network of polygonal cells in reticular arrangement, adhering to each other only by thin, sometimes branching cytoplasmic processes (fig. 5). In some places, however, especially near the explant, the cells lay closer together, so that the membranes had a more continuous appearance. In the peripheral parts the cells assumed a more outstretched and spindle-shaped form. Between the cells were large and quite irregular gaps which became progressively wider towards the periphery.

The cytoplasm of the cells was slightly basophilic. The nuclei were roundish or oval, irregularly outlined and rich in chromatin. Nucleoli were not always distinguishable (fig. 6). Mitoses were present but scanty.

After further incubation in Carrel flasks (16 to 18 days) the outgrowing membranes became somewhat denser (fig. 7). They consisted mainly of polygonal cells lying closely together (fig. 8). In these membranes, however, as contrasted with the membranes developing from lung explants without visceral pleura, pavement formation never occurred. In addition to the membranes just described, in lung fragments explanted with visceral pleura, membranes of epithelial cells in pavement formation could be seen at some isolated points. They were very small and in comparison with the large membranes mentioned above seemed insignificant.

Thus the main membranal outgrowth from lung fragments explanted with visceral pleura showed all the characteristics

known for mesothelial cells in vitro. It presented the same aspect as the cell sheets in cultures of serous membranes described by Lewis, Maximow and Chlopin.

II. Observations on the proliferative changes occurring in the interior of the explanted lung fragment

After 24 and 48 hours of incubation the characteristic structure of the lung remained fairly well preserved. The alveoli were of normal size and form, but some of them appeared collapsed. The alveolar walls were thin and not very cellular. Two cell types could be distinguished in the septa of the alveoli: spindle shaped cells with scanty cytoplasm and elongated nuclei fairly rich in chromatin. These cells were relatively numerous and equally distributed throughout the septa. The others were roundish, with abundant clear cytoplasm, and spherical or oval nuclei containing little chromatin and 1 to 2 clearly visible nucleoli. These cells were irregularly spaced and at some distance from each other. In some places they bulged into the alveolar lumina. A certain number of cells of the latter type could also be seen free in the lumina of the alveoli. Here their cytoplasm was often vacuolated and, after staining with Giemsa's stain, displayed 2 distinct zones: an internal pink and an external bluish one. Nuclear debris and red blood corpuscles could sometimes be seen in their cytoplasm.

In lung fragments examined after 48 hours of incubation the roundish cells of the alveolar septa, which previously appeared scattered, and were always at some distance from each other, could sometimes be found in small groups, many cells being in mitosis (fig. 17). Small bronchioli, which were occasionally met with, were well preserved after 24 and 48 hours. Mitosis could occasionally be seen in their epithelial lining. Detached cells within the bronchiolar lumen were rarely seen, and in no case did they contain phagocytized material.

The lung fragments explanted without visceral pleura had no cellular lining on the surface of the explant 48 hours after

explantation. Lung fragments explanted with visceral pleura became at this time surrounded by a thin layer of elongated cells which covered the surface of the explant. The cytoplasm of these cells was basophilic and sometimes vacuolated; their nuclei were fairly rich in chromatin and sometimes wrinkled. Some of these cells were swollen and, here and there, formed budlike formations protruding into the periphery. The origin of the described cells from the pleural covering of the lung can be clearly traced. The plasma surrounding the lung fragments explanted with visceral pleura was liquefied, and contained, in addition to few leucocytes, large spherical cells similar to those exfoliated into the alveolar lumina, and smaller outstretched or polygonal cells with scarce cytoplasm and nuclei rich in chromatin. The latter type of cells were derived from the pleura, and were not present in the periphery of lung explants without visceral pleura.

After 72 hours of incubation the most striking change noted was an enlargement of the alveolar septa with marked increase in their cell content, especially of the big, roundish cells protruding into the lumina of the alveoli. Cells of this type formed rows lining the alveolar lumina for a short stretch. Here and there budlike formations of these cells were seen. Mitoses were frequent. In alveoli small irregularly arranged cell sheets, consisting of polygonal cells with abundant, slightly basophilic, often vacuolated cytoplasm, could sometimes be found (fig. 9). The nuclei of these cells showed a great resemblance to those of the proliferating cells of the septa. In some places the continuity of the cells forming the intraalveolar sheets with those lining the alveoli could be followed.

At the periphery of lung fragments without visceral pleura membranal formations consisting of large oval or elongated cells lying close together could be observed. The cytoplasm of the cells forming these membranes was, like that of the cells protruding into the alveoli, markedly basophilic and their nuclei were poor in chromatin and contained 1 to 2 dark nucleoli. In the zone of liquefaction numerous free spherical

cells of the same type, frequently containing a distinct eosinophilic centrosphere were found. They were identical with the cells exfoliated into the alveoli.

Lung fragments containing visceral pleura were, here and there, bounded by rows of elongated cells deriving from the pleural lining and similar to those described after 24 and 48 hours of incubation. At some points in the periphery these cells formed a delicate tissue, the individual cells being connected with each other solely by very thin cytoplasmatic processes as in the mesothelial membranes described above. The same type of cell was encountered in the zone of liquefaction.

After 96 hours of incubation the cells lining the alveoli were found to be in a state of intense proliferation. Here and there the alveolar septa consisted almost entirely of roundish voluminous cells, whereas the other cell components of the septa were inconspicuous (fig. 10). Large budlike formations of these roundish cells were noted jutting into the alveolar lumina. Some of the alveoli were completely invested by a single layer of cubical cells, thus having a glandlike aspect (fig. 11). In the subpleural areas the marginal alveoli were lined with continuous ribbonlike indented layers of cubical cells, which extended from alveolus to alveolus (fig. 12). At the same time in some areas large fanlike membranes could be seen filling the lumina of the alveoli. The membranes consisted of large cells lying close together. The cells had basophilic, often vacuolated cytoplasm, and nuclei of varying size, often eccentrically located (fig. 13). At the periphery of the explants both with and without visceral pleura, the same type of outgrowth was noted as after 72 hours, but at this time the outgrowth of common fibroblasts could also be observed.

After 120 hours of incubation the cell sheets filled the alveolar lumina so completely that large portions of the section had the appearance of solid tissue (figs. 14, 15). The alveolar wall consisted, for the most part, of cubical or polygonal cells often arranged in rows, so that, where alveolar lumina were present, they had a glandlike appearance. Where

the alveoli had collapsed, the septa, tightly packed with cells, lay close together. In some places the boundaries of the cells of the alveolar septa were indistinct, the cells coalescing into syncytia. The nuclei were abundant, varying in size and mitotic divisions were often seen (fig. 16).

The outgrowth of the cells in the periphery of the explant was of the same character as that noted after 72 and 96 hours, but covering greater areas. Here and there membranelike formations growing into the plasma could be seen.

DISCUSSION

The problem of the origin and character of the cells lining the pulmonary alveoli is the object of great divergence of opinion. Up to the present day no agreement has been reached as to whether these cells should be regarded as epithelial or as mesenchymal elements. A number of authors maintain that the cells lining the pulmonary alveoli are epithelial cells of entodermal origin, whereas other investigators assume that they are mesenchymal. A thorough discussion of this problem from different points of view is given by Seeman, Fried, Bargman, Clara, Macklin, Miller, Loosli and Policard.

Various methods were used in the investigation of the nature of the cells lining the pulmonary alveoli. Many investigators founded their assumptions on purely histological methods, studying the morphological aspects of the cells lining the alveoli and their topographical relation to the other cell elements of the alveolar wall. For that purpose the method of silver impregnation in various modifications was primarily used. Some authors tried to solve the problem by means of vital staining, studying the behavior of the lining cells of the pulmonary alveolus with respect to the injected dyes. Other authors used the method of tissue culture. This method appears especially promising, because it offers the opportunity of studying not only the morphological and functional properties of the cells but also their prospective potencies.

There is an almost general agreement that explants of lung tissue in vitro may give rise to an outgrowth of cells forming continuous membranes around the explant. These membranes have an essentially epithelial character. Most of the discussion has centered upon the question of the cell type from which the outgrowth of the epithelial membranes arises. On the one hand, it was claimed that the cell membranes develop from the epithelium of the bronchioli or from the lining cells of the pleura, and that they have no connection with the cells lining the alveoli. On the other hand, it was assumed, that the outgrowing epithelial membranes derive from the cells lining the alveoli and from these alone.

Carleton was the first to observe the outgrowth of epithelial membranes in lung cultures from full term cat embryos. In serial section he demonstrated that the cells forming the epithelial sheets were continuous with the lining cells of an alveolus. On the other hand the lung explants were often covered by an epithelial layer of another type which the author designated as "cicatricial epithelium" and which he believed to derive from the cut edges of the bronchi.

Caffier referred to the formation of epithelial membranes in explants of lungs from human embryos of the third month as a very rare exception. These membranes, according to the author, may possibly derive from "bronchial structures" of the explant. More frequently the explant was surrounded by an "aureole" the cells of which had at first an epithelium-like aspect but assumed fibroblast-like character, when further cultured. He thought that this type of outgrowth may originate from the lining cells of the pleura.

Verne described membranes of typical epithelial aspect in cultures of lungs from chick embryos. The author stressed the fact that they did not derive from the pleura, since exactly the same membranes were observed in lung fragments which were free from pleura.

Lang in cultures of lungs from adult rabbits never saw the outgrowth of epithelial membranes. The only kind of epithelial development he observed was of the type described by Carleton as "cicatricial epithelium" which covers the surface of the explant in a continuous layer and which, according to the author, derives from the epithelium of the bronchi.

Considering the scarcity of the observations and the discrepancies among the conclusions drawn from them, it seemed worthwhile to repeat the experiments on cultivation of lung tissue in vitro. In doing so, the outmost care was taken to study the various types of epithelial growth taking place in and around the explant, as well as to trace its origin.

The experiments reported in this paper showed that an outgrowth of epithelium-like membranes regularly occurred in cultures of lung fragments. The structure of the outgrowing membrane varied, depending on the origin from lung fragments with or without pleura. In explants free from visceral pleura, the outgrowing membrane represented a dense sheet-like formation. It consisted of polygonal cells adhering closely to each other along their edges. Since the intercellular boundaries were generally visible, the membrane showed a mosaic pattern. At no point, even at the extreme periphery, did the cells show the least resemblance to fibroblasts. The appearance of this formation was that of a typical epithelial membrane.

If, however, lung fragments with visceral pleura were explanted, the character of the outgrowing membrane was quite different. In places such membranes had an epithelial aspect and resembled the membranes described above; but in most cases the same outgrowing membranes displayed a delicate netlike pattern. The cells forming these membranes were chiefly polygonal cells, separated from each other by more or less large gaps and were connected only by thin irregular branching processes. At the edges of the membrane the cells became more outstretched and resembled common fibroblasts. These netlike membranes developing from lung fragments with visceral pleura show all the characteristics of mesothelial growth, as described by various authors. In addition to the net-like membranes surrounding the whole explant in some cultures a slight outgrowth of typical epithelial membranes, consisting of cells in pavementlike arrangement was noted at isolated points. These membranes probably originated from lung edges not covered by visceral pleura.

Membranes of exactly the structure discussed above were described by Lewis in explants of pericardium, by Maximow in explants of mesentery and of the peritoneal surface of the centrum tendineum of the diaphragm, and by Chlopin in explants of mesentery and pericardium. Maximow points out that the lining cells of the serous membranes, originating in the coelomic epithelium, although histologically of an epithelial character, have prospective potencies of a double nature — epithelial and fibroblastic. In cultures, therefore, these cells may show transitory epithelial growth. In the course of time, however, the mosaic pattern of the membranes disappears and fairly large gaps develop between the indvidual cells; the cells themselves assume a polymorphic character and adhere to one another by very thin processes. They gradually grow more and more outstretched and eventually transform into fibroblasts.

From the reported observations it seems plain that typical epithelial growth arises from lung explants without visceral pleura, whereas typical mesothelial membranes can be observed in lung explants with visceral pleura; these latter membranes obviously develop from the cells forming the pleural lining.

What is the origin of the epithelial growth developing from lung fragments without visceral pleura? This question can be clarified only by correlated studies of the growth processes taking place outside and inside of the explanted lung fragments.

Carleton cultured lung fragments from adult rabbits and cats in plasma tubes and studied them in serial section. He observed swelling and detachment of the alveolar epithelium as well as stimulation of the mitotic activity of these cells. He further reported that "the adult lung in vitro rapidly dedifferentiates and its elements become strikingly foetal in appearance, owing to the small alveolar cavities and the thickened epithelial cells lining them."

Lang studying explants of rabbit lungs, also reported epithelial growth in the interior of the explanted fragments. He even described "carcinomalike formations." In his opinion, however, any kind of epithelial growth in lung explants originates from the epithelium of the bronchi.

Timofejewski and Benevolenskaja reported proliferation of alveolar epithelium in epithelial arrangement in explants of rabbit lungs to which Tb-bacilli were added.

In our experiments histological examination of the lung fragments surviving in plasma revealed an intense proliferation of epithelial cells inside the explant. That this growth originated in the lining cells of the pulmonary alveoli became evident from the study of serial sections of fragments incubated for various periods of time. Already after 48 hours of incubation numerous mitoses were noted in the lining cells of the alveoli, the number of these cells being obviously increased. These cells, which were previously scattered and at some distance from one another formed short rows after 72 hours. In some places there were extensive papillary projections into the lumina from the cells lining the alveoli. At other points the cells lining the alveoli proliferated in the form of small cell buds penetrating into the alveolar lumina. After 96 and 120 hours of incubation the proliferating cells of the alveolar lining fused into continuous membranes which partially or completely obliterated all the alveoli. The lung fragments thus transformed into a solid tissue. Side by side with this sheetlike growth filling the alveoli, another type of epithelial growth originating in the alveolar lining, was noted. The cells lining the alveoli occurred as a continuous layer of cubical cells investing the alveolar lumina, which thus appeared as glandlike formations. Where the alveoli were collapsed the septa appeared as solid epithelial bands separated only by narrow gaps. The boundaries of the cells of the alveolar walls were frequently indistinct.

We do not know why in one case the epithelial sheets are formed filling the alveoli, whereas in another case the formation of continuous ribbonlike epithelial cell layers investing the alveolar lumina can be seen. It is likely that we are dealing with 2 stages of the same process, but it may also be that variations in the environmental conditions, e.g. the alveoli being empty or filled with plasma, determine the pattern of epithelial proliferation.

However that may be, it is quite certain that both types of epithelial growth taking place in the interior of the lung explant derive from the lining cells of the alveoli.

There is no doubt that the epithelial membranes developing outside the explanted lung fragment are of the same nature and origin as the abundant proliferation in the interior of the explant. This was confirmed by the study of serial sections from lung explants of 72 to 120 hours of incubation. These sections revealed that the epithelial outgrowth observed in the plasma derived continuously from the lining cells of cut alveoli, situated at the periphery of the explanted lung fragment. Thus the relevant observations of Carleton, mentioned above, were confirmed.

The possibility cannot be excluded that the lining cells of the bronchioli respiratorii may also participate in some places in the formation of sheets and membranes. However, as could be clearly shown in serial section, the major part of the epithelial growth taking place in lung explants in vitro occurred entirely independent of bronchi.

In conclusion it may be said, that the epithelial growth occurring around and in the interior of explanted lung fragments derives primarily from the cells lining the pulmonary alveolus. Since the lining cells of the alveolus give rise to typical epithelial growth, it must be concluded that they themselves are epithelial and not mesenchymal cells.

Finally it seems worthwhile to mention that both types of proliferation of alveolar epithelium, namely the formation of (a) intraalveolar epithelial sheets and of (b) continuous layers of cubical cells lining the alveoli, as seen in cultures, are very similar to those occurring in the lung under various pathological conditions. In the following a few pathological findings are referred to, owing to their remarkable resemblance to those observed in vitro.

After the compression of the lung by pneumothorax the alveoli in the human lung (Muralt) as well as in the lung of rabbits (Dogliotti and Amprino) were found to be lined with a continuous layer of cubical epithelium (Muralt, fig. 11,

p. 19). A similar result was obtained after the injection of oily substances into the pleural cavity (Wenslaw, figs. 1 and 2, pp. 864 and 865; Tschistowitsch). Young produced hyperplasia of alveolar epithelium in the alveoli bordering the visceral pleura by the injection of electrolyte solutions into the pleural cavity. One of the microphotographs given by Young is very similar to figure 12 of this paper (Young, fig. 15, plate 36). In circulatory failure owing to mitral stenosis Parker and Weiss noted that the walls of the alveoli were covered with cubical cells (Parker and Weiss, fig. 7, plate 109). Proliferation of the lining cells of the alveoli was also reported in acute and chronic inflammatory processes. In his book "The Lung" Miller gives an illustration showing how the cells of the alveolar septa are pushed off as continuous sheets in pneumonia (Miller, fig. 47, p. 63, compare with fig. 9 of this paper). According to Cowdry in Jagziekte of sheep the alveolar epithelium shows proliferation with penetration into the alveolar lumen (Cowdry, figs. 6 and 9, plate 18). The filling in of alveoli with epithelial membranes has been described by Bargmann (fig. 13, p. 821) for the hedgehog and was observed by the author in the lungs of rats treated with repeated doses of urethane. In recent papers Bell as well as Geever, Neubuerger and Davis draw attention to the common occurrence of proliferation of alveolar epithelium (Bell) or of septal cells (Geever, et al.) in pathological processes of the lungs.

It is probable that the same mechanism is instrumental in the production of the described changes in vivo and in vitro. Aschoff already pointed out that alveoli situated in parts of the lobule where respiration is less intensive, or alveoli filled with fluid, are often lined with discontinuous epithelial layers. Failing respiratory movement or the filling in of the alveoli by plasma or serous fluids are factors present in vivo, in diseased lungs, as well as in vitro, in lung explants. It appears, therefore, quite obvious that under both conditions growth may occur along similar lines.

SUMMARY

1. Cultures of lungs from adult rabbits show membrane-like outgrowth around the explant. The outgrowing membranes are of epithelial character if they originate from lung fragments without visceral pleura, and of mesothelial character, if they originate from explants with visceral pleura.

2. Epithelial growth also takes place in the interior of the explanted lung fragment. This epithelial growth, as ascertained by serial section, originates in the lining cells of the alveoli. The alveoli of the explanted lung tissue are frequently surrounded by a continuous layer of cuboidal epithelium or filled with epithelial sheets.

3. These findings suggest that the lining cells of the pulmonary alveolus giving rise to epithelial growth are themselves of epithelial nature.

4. The resemblance between proliferations of the lining cells of the pulmonary alveolus under pathological conditions and the proliferative processes observed in vitro are discussed.

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PLATE 1

EXPLANATION OF FIGURES

1 Lung culture. Hanging drop. Nine days of incubation. Epithelial membrane outgrowing from a lung fragment explanted without visceral pleura. Giemsa stain. × 38.

2 The same as culture 1. Cells of the epithelial membrane in pavement formation. Mitotic divisions. × 580.

3 Lung culture. Carrel flask. Fifteen days of incubation. Epithelial membrane outgrowing from a lung fragment explanted without visceral pleura. Pavement arrangement of the cell forming the membrane. Living. × 43.

4 An area of the same culture. × 130.

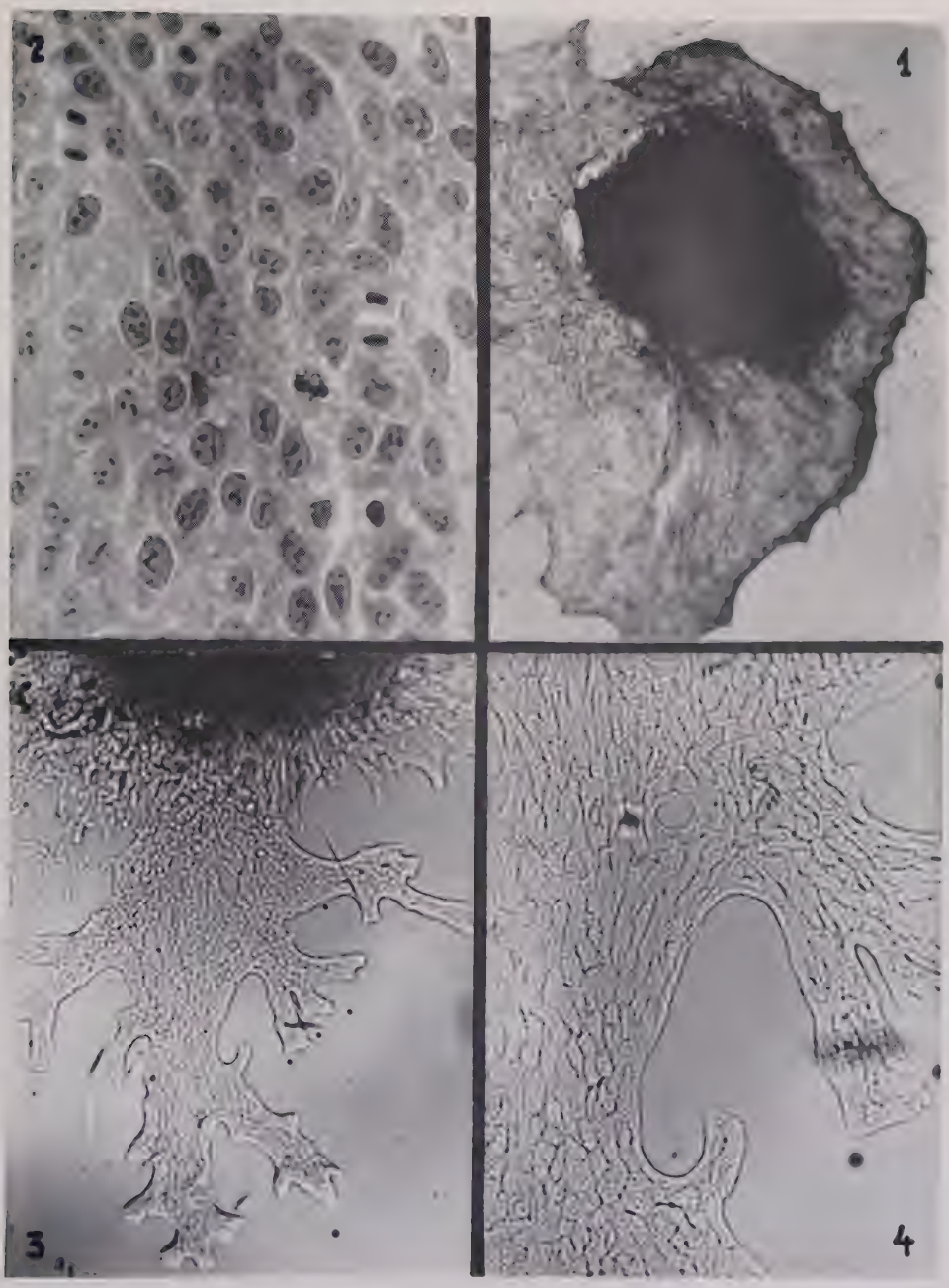
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PLATE 2

EXPLANATION OF FIGURES

5 Lung culture. Hanging drop. Eight days of incubation. Mesothelial membrane outgrowing from a lung fragment explanted with visceral pleura. Giemsa stain. × 85.

6 The same culture as 5. Cells of the mesothelial membrane adhering to each other by branching processes. × 590.

7 Lung culture. Carrel flask. Eighteen days of incubation. Mesothelial membrane outgrowing from a lung fragment explanted with visceral pleura. Reticular arrangement of the cells forming the membrane. Living. × 45.

8 An area of the same culture. × 90.

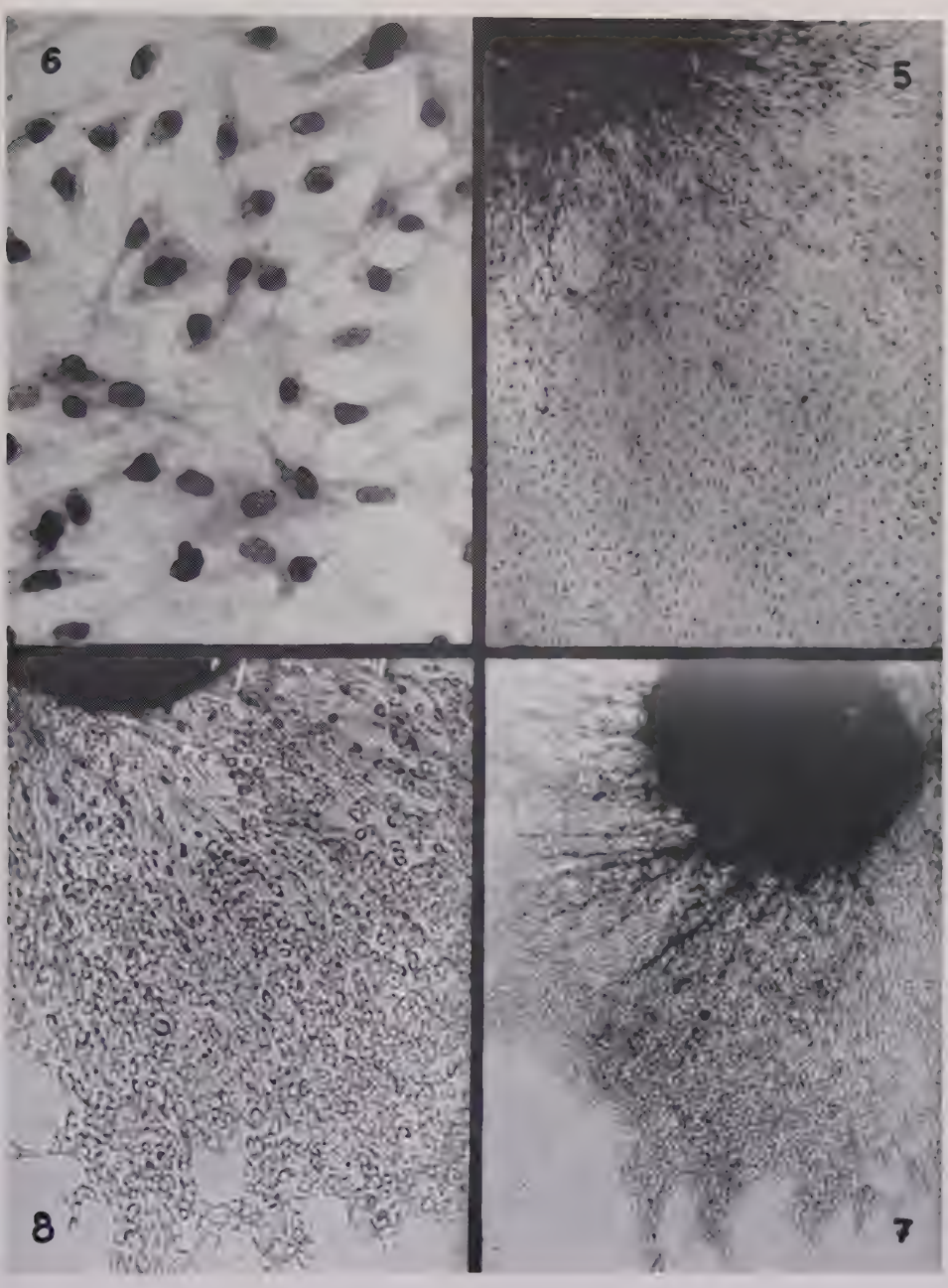
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PLATE 3

EXPLANATION OF FIGURES

9 Section from lung culture. Seventy-two hours of incubation. Alveolar septa enlarged. Large roundish cells protruding into the alveoli. Cell sheet in the lumen of an alveolus. Giemsa stain. × 690.

10 Section from lung culture. Ninety-six hours of incubation. Intense proliferation of the lining cells of the alveoli. Mitotic divisions. Giemsa stain. × 1500.

11 Section from lung culture. Ninety-six hours of incubation. Alveoli invested by layers of cubical cells. Giemsa stain. × 590.

12 Section from lung culture. Ninety-six hours of incubation. Subpleural alveoli lined with continuous ribbonlike indented layers of cubical cells. Giemsa stain. × 590.

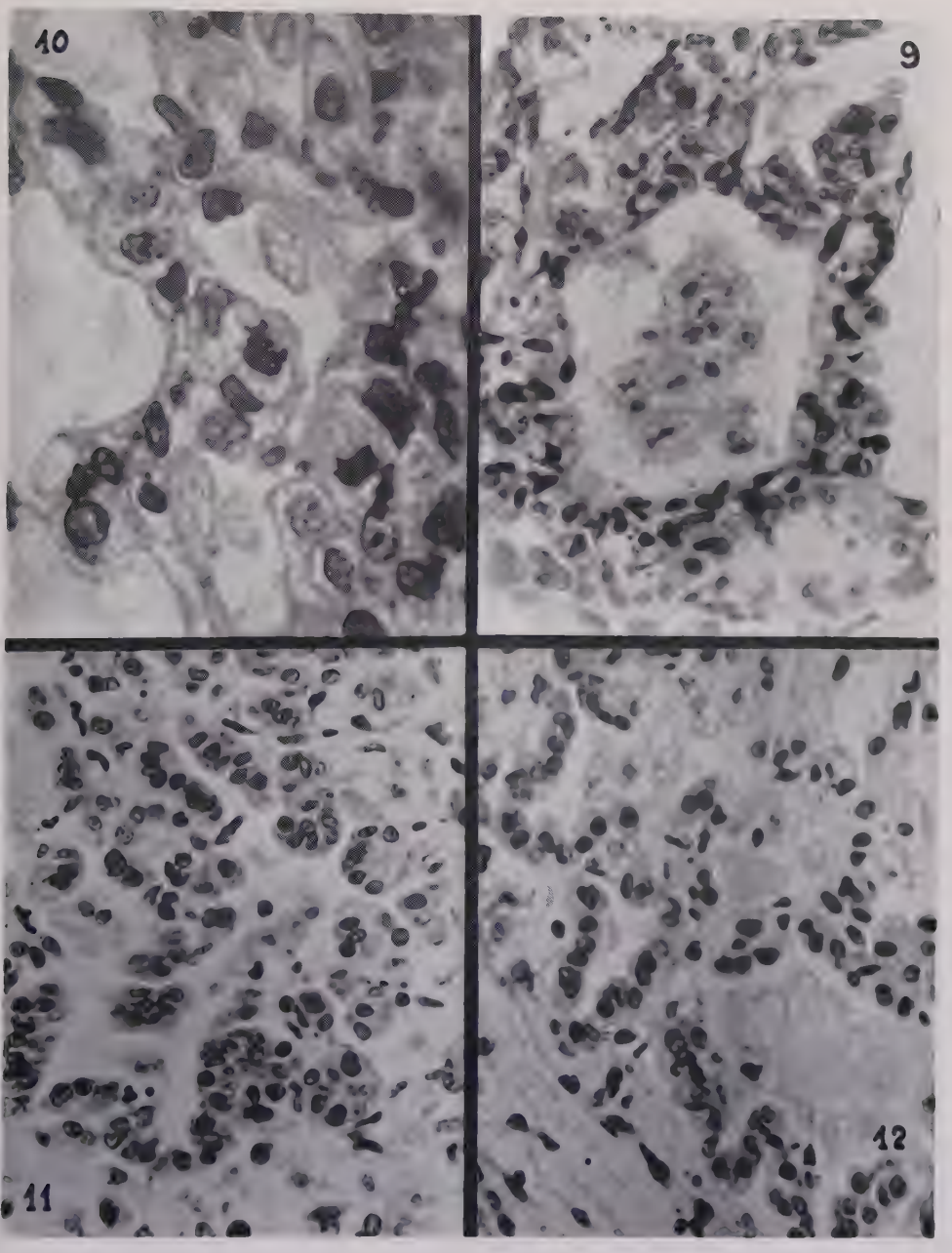
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PLATE 4

EXPLANATION OF FIGURES

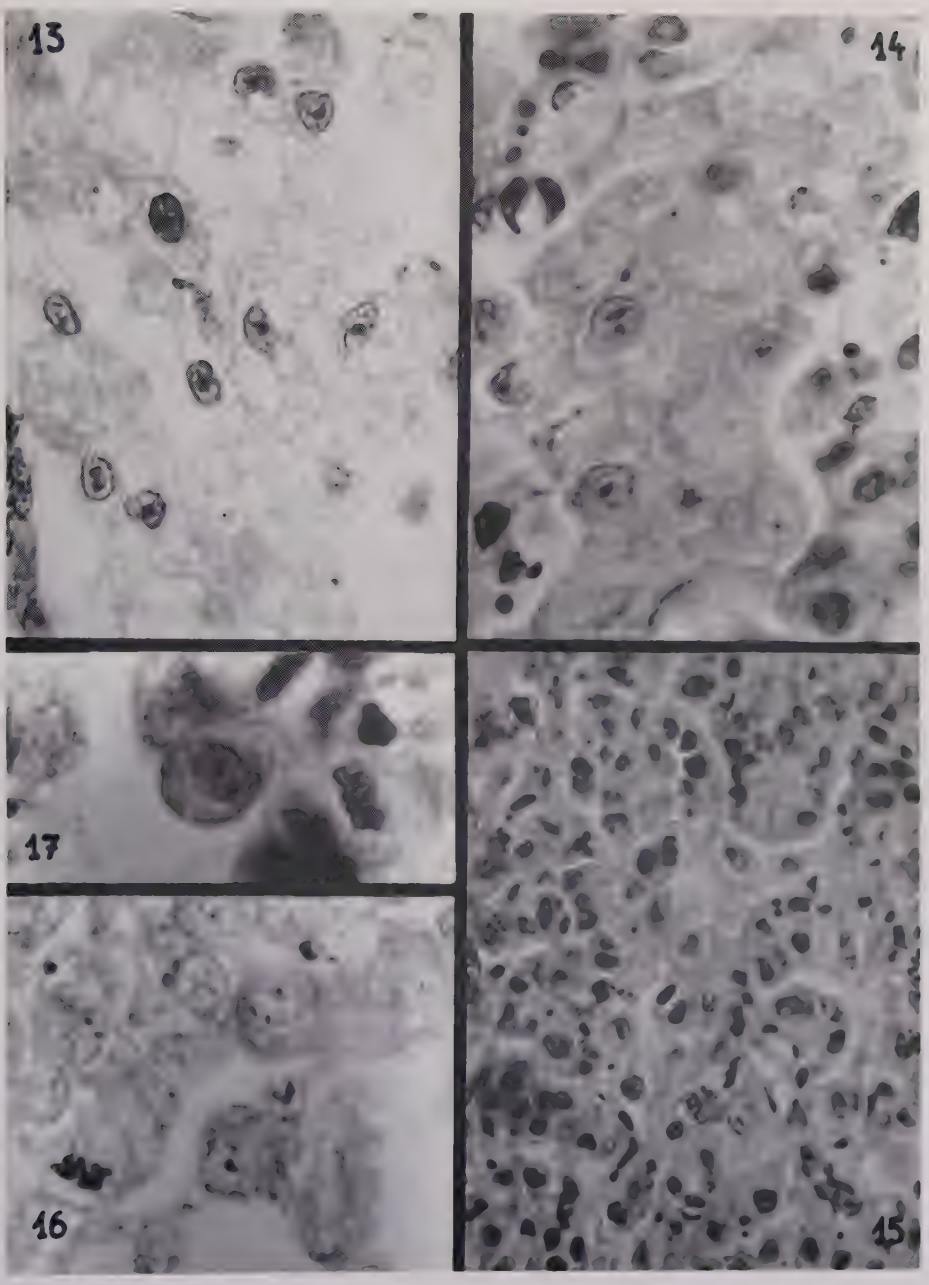
13 Section from lung culture. Ninety-six hours of incubation. Intraalveolar cell sheet. Giemsa stain. × 1320.

14 Section from lung culture. One hundred and twenty hours of incubation. Intraalveolar cell sheet filling the alveolar lumen completely. Giemsa stain. × 1320.

15 Section from lung culture. One hundred and twenty hours of incubation. Lung fragment showing the appearance of solid epithelial tissue. Giemsa stain. × 670.

16 Section from lung culture. One hundred and twenty hours of incubation. Large roundish cells of alveolar septa in intense proliferation. Mitotic division. Giemsa stain. × 1400.

17 Section from lung culture. Forty-eight hours of incubation. Small group of roundish cells bulging from the alveolar wall into the alveolar lumen. Giemsa stain. × 2000.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MITOTIC RATE IN MOUSE LIVER FOLLOWING INTRAPERITONEAL INJECTION OF LIVER, KIDNEY AND EGG YOLK

J. WALTER WILSON AND ELIZABETH H. LEDUC
Arnold Biological Laboratory, Brown University, Providence, Rhode Island

FOURTEEN FIGURES

INTRODUCTION

In the liver of the adult mammal mitotic figures are so rare that they are usually said to be absent. Dawson ('40) has stated that 1 cell in 10,000 to 20,000 may be active. Nevertheless, the tissue is capable of very rapid growth by active mitosis. This occurs after some injuries, as for example necrosis following phosphorous or chloroform poisoning, or in regeneration after removal of a part of the organ. The problem that this initiation of mitotic activity in a quiescent organ presents is a particular case of the much larger and more general problem of the cessation of growth in the organism or its tissues and its resumption at a time of need.

This paper presents some experiments performed in an attempt to stimulate mitosis in the mouse liver. As long ago as 1899 Mathews attempted to stimulate mitosis by the injection of alkaline extracts of organs under the assumption that increase in nucleic acid would accelerate the synthesis of chromatin and thus permit cells to divide faster. If the rate of synthesis of nucleic acid is a limiting factor in mitotic rate he should have obtained an increased number of mitoses, which he believed he did. McJunkin and Breuhaus ('31) obtained an increased number of mitoses in regenerating rat liver by intraperitoneal injections of pulped liver. They also produced a slight amount of mitosis in normal adult rat livers by the

same method. If the products of autolysis of the necrotic area around the stumps of amputation are the stimulating agents for liver regeneration this would be expected. Marshak and Walker ('45) obtained increased numbers of mitoses in regenerating rat livers by the intravenous injection of liver nuclei and various fractions of them. Weiss and Wang ('41) by making grafts of liver onto the area vasculosa in the chick embryo, obtained enlarged livers in the host chicks, and found an increased mitotic index in their livers. Since the grafted liver does not grow, they concluded that some specific organ discharge of the liver carried to the host through the circulation was the stimulating agent. On the basis of these suggestive observations we have attempted to test the effect of various materials on the rate of mitosis in young mice of 3 weeks, in which a small amount of mitosis is normally found, and also in young adults in which mitosis has usually entirely ceased.

MATERIALS AND METHODS

The mice used in these experiments were of a Swiss strain raised in this laboratory from stock obtained from Albino Farms in Red Bank, N. J. They were fed Rockland Mouse Pellets, occasionally supplemented with milk, carrots and lettuce. For the most part they have bred well and in growth and general appearance have seemed normal and healthy. This is to be emphasized because we have sometimes found focal necrosis in livers of both experimental and control animals. At first we considered this to be due to a mild infection of mouse typhoid, but the animals showed no signs of disease and we were unable to isolate the bacteria from them. It is possible that the necrosis is due to dietary inadequacy. This seems improbable to us, however, since even when supplements are added to the Rockland pellets, which we have in general found to be adequate alone, the focal necroses are occasionally found. It is possible that some of the sporadic results which we have obtained may be due to the action of the agent causing this focal necrosis. Olitsky and

Casals ('45) have described such lesions in livers of stock Swiss mice which were otherwise perfectly normal and healthy in appearance, and they have been unable to ascribe them to any definite causative agent. Since we have found many animals with focal necrosis but with no mitotic activity in the liver, we have continued the experiments.

In most of the experiments, a family of mice of the same age that had been kept together was used. One or more were used as controls and the rest received intraperitoneal injections of the substance to be tested. They were then killed at regular intervals and the mitotic activity of their livers was determined. They were killed by a blow on the head followed by decapitation and bled as completely as possible. A thin slice (2–3 mm) of the large left lateral lobe of the liver was fixed in Bouin's fluid and imbedded in paraffin through dioxan. Sections were made at 5 micra and stained in Delafield's haematoxylin and eosin.

A figure representing the mitotic activity was computed from the following 3 numbers: The number of mitotically active cells in a section counted under an oil immersion lens at 1125 ×; the area of the section, measured by a planimeter on an outline drawn from a projected image of the section; the nuclear density of the section, determined by averaging the number of nuclei counted in several fields selected so as to contain no large blood vessels. The mitotic activity was then computed according to the following formula:

$$\frac{\text{Number of mitotically active nuclei}}{\text{Area of section} \times \text{nuclear density of section}} = \text{mitotic activity of the liver}$$

This is obviously an arbitrary number but it serves adequately as a comparative measure. To have extended the computation further to determine the mitotic index, that is, the number of mitotically active cells per thousand, would have implied a degree of precision that our procedure would not warrant.

The counting of the nuclei in mitosis is not a simple matter. Duplicate counts made on the same section at different times

may show a surprising variation. This is due in part to the fact that there is no clearly defined line of demarcation between the resting nucleus and one in prophase, or between a late telophase and a resting nucleus. It would be desirable in some ways to use thicker sections, but since the counting to be accurate must be done with an oil immersion lens, the travel of the fine adjustment would then be so great as to make the counting tedious.

The mitotically active nuclei of hepatic cells alone were counted. Those of the lining cells of the sinusoids and of the duct cells were ignored. In order to obtain a more complete picture the mitotically active nuclei were classified as to phase as they were counted. Among other things it was thought possible that the total number might bear a constant relation to some easily recognized phase, such as the metaphase, so that the work of counting could be simplified. This did not prove to be the case. The relative proportion of the various phases varied greatly in different animals, so much so that we have considered the possibility that mitosis sometimes occurs in waves rather than at a constant rate.

A series of counts made by both of us on a single section indicated that the procedure is sufficiently accurate, especially when the variability of the material is taken into consideration. Similarly, counts of sections from the different lobes of the same liver showed good correspondence.

When the count was completed an eyepiece with a Howard disk was inserted so that the field was marked off in squares. The nuclei of the parenchymal cells were then counted with an automatic counter. In this case each nucleus of a binucleate cell was counted as one, and each mitotic figure as one. Fields were selected so that no large vessels were included. Several fields from different parts of the section were counted and the numbers averaged. This average is the "nuclear density." In most specimens the variation in different parts of the section was not very great, but in different animals it may be very great. Animals have been found with nuclear densities as low as 30 and as high as 130. The number decreases with

age, and is also small when the cells appear to be highly vacuolated.

The materials to be injected were obtained sterilely and ground in a simple tissue grinder made by plunging a glass rod with melted end into a pyrex test tube as described by Potter and Elvehjem ('36) or in a commercial tissue grinder.[1] Sufficient sterile Ringer's fluid was added to give the material a suitable consistency for injection. Precautions were taken to keep the procedure sterile. Glassware, syringes, needles and saline were sterilized in the autoclave and dissecting instruments were sterilized in alcohol. The surface of all animals was scrubbed with alcohol at the time of injection. All injections were made intraperitoneally.

OBSERVATIONS

Injection of homologous liver into young mice. A group of experiments (fig. 1) was conducted to test the effect of injections of homologous liver into young mice, approximately 3 weeks of age. All control animals showed a small amount of mitosis in the liver.

The liver to be injected was obtained from adult mice of uncertain age. The entire liver, minus the gall bladder, was removed and thoroughly ground with 2 cm^3 of sterile Ringer's solution. 0.5 cm^3 of this pulped liver was injected into each experimental animal by means of a tuberculin syringe and a 20 gauge needle.

In each experiment one litter of animals was used. One animal was killed as a control and the rest were injected. The injected animals were then killed at intervals following injection and the mitotic rate in their livers was determined. In experiment 23 (fig. 1) six 19-day-old mice were injected and then killed on the third, fifth, eighth, tenth, thirteenth and sixteenth days after injection. Twenty-day-old animals were used in experiment 30 (fig. 1) and were killed every other day from the third to the eleventh days after injection. In experiment 31 (fig. 1) the experimental animals were 21 days of age when

[1] Manufactured by the Scientific Glass Apparatus Company, Bloomfield, N. J.

injected and they were killed on the third through the tenth days following injection. Each point on the curves represents the mitotic rate observed in one animal.

In each experiment a peak of mitotic activity which greatly exceeds the mitotic rate in the control animals is obtained approximately 7 or 8 days after injection. A decrease from normal mitotic activity consistently appears during the first

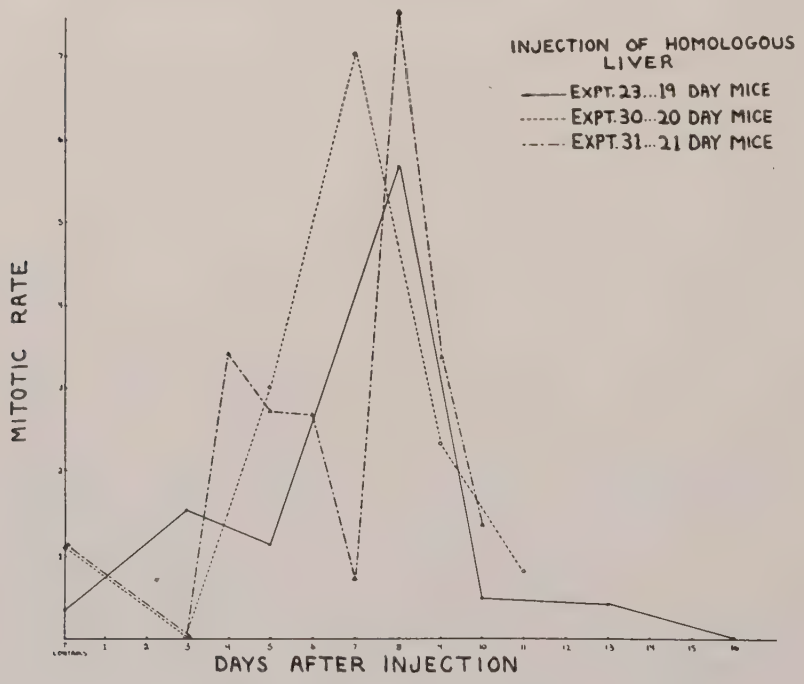

Figure 1

few days after injection. Irregularities in the graph, such as sporadic increases, e.g. experiment 31, fourth day (fig. 1), or decreases, e.g. experiment 31, seventh day (fig. 1), are difficult to explain. McJunkin and Breuhaus ('31) have reported animals with similar unexpected numbers of mitotic figures which they were unable to explain. How long the induced wave of activity lasts in each mouse is, of course, not known, but it is possible that if the course of events in a single animal could

be followed, a regular graph would result, and that the irregularities of these experimental graphs are due to the fact that the peak does not appear simultaneously in different animals.

Injection of homologous liver into adult mice. Injections of homologous liver similar to those above were given to young adult mice in which mitotic activity in the liver had ceased. It was felt that an initiation of mitosis in adult, mitotically inactive tissue would be of even greater significance than the increase in rate where mitosis was already present.

The results of 2 such experiments are presented graphically in figures 2 and 3. Both the donors and the experimental animals in experiment 25 (fig. 2) were young adults of uncertain

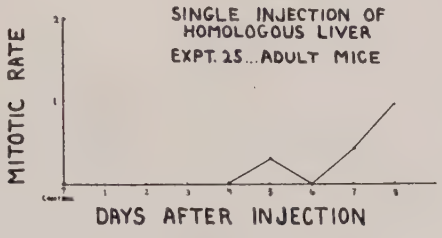

Figure 2

age. The homologous liver was prepared as described above and 0.5 cm^3 was injected into each experimental animal. Two of these animals were killed on each of the fourth to the eighth days after injection. One mouse was killed as a control on the day of injection. No mitotic figures were found in its liver.

In experiment 50 (fig. 3) a group of 16 young adult mice from 2 litters, 1 of 12 and 1 of 10 animals, 8 weeks of age, were injected with mouse liver. The liver was obtained from 5 adults of a third litter of the same age and prepared in the usual way. Eight of the injected animals from 1 litter (series A) received an injection of 1 cm^3 of the ground tissue or roughly one-third of a liver. The 8 injected mice of the other litter (series B) received 0.5 cm^3 or approximately one-sixth of a liver. Two animals, one from each series, were killed

each day after injection. The remaining 6 mice from these 2 litters served as control animals. Two were killed on the day of injection and 2 on the fourth and 2 on the eighth day after injection. No mitotic activity was found in any of the controls. In this and in other experiments we have found no

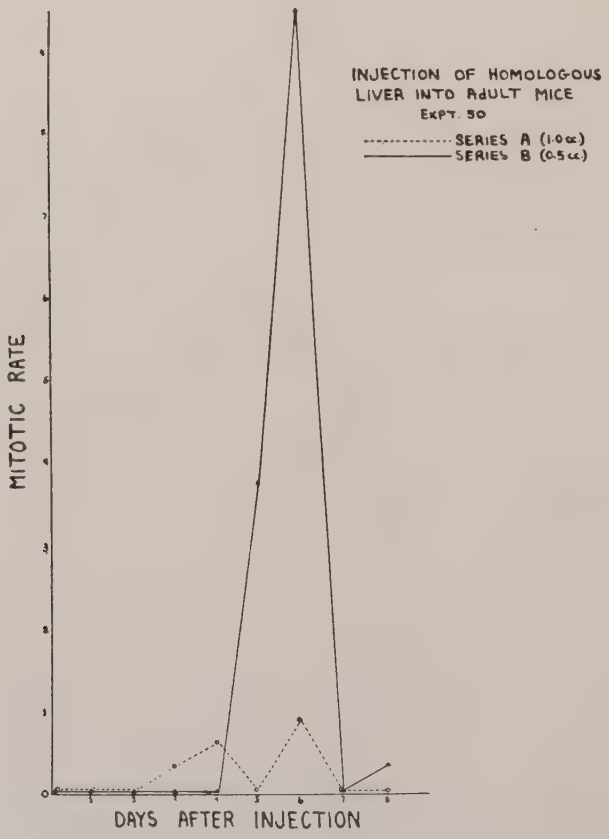

Figure 3

consistent quantitative relationship between the amount of material injected and the amount of mitosis induced.

In general, the peaks of mitotic activity in the adult mice were lower than those observed in the younger animals. Nevertheless, they seem more significant in view of the fact

that no mitosis was present at the start. Although no initial depression could be observed in the adults, the peaks of activity occurred at approximately the same time as in the younger mice.

McJunkin and Breuhaus ('31) obtained no mitotic activity in the adult rat after a single injection but did obtain it after a second injection given 4 days after the first. Following this procedure, 2 groups of adult mice were given 1 injection of 0.5 cm^3 of homologous liver which was then followed by another of equal amount 4 days later (fig. 4). The wave of mitosis appeared at the proper time after the second injection.

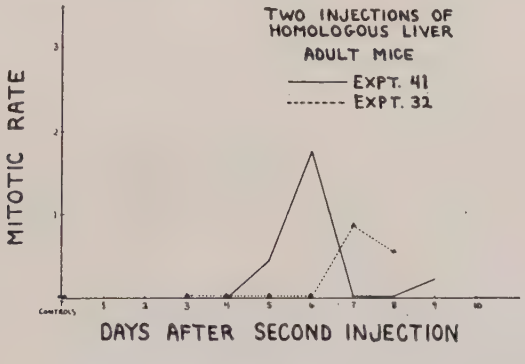

Figure 4

There was no additive effect. The effect of the first injection seems to have been suppressed.

Injection of perfused mouse liver. In order to minimize as much as possible any effects which might be produced by the residue of blood in the donor tissue, 1 experiment was conducted in which the liver was perfused with saline before it was removed from the animal.

The donor liver was perfused by injecting sterile 0.9% saline into the portal vein by means of a tuberculin syringe and a 27-gauge needle until the color of blood no longer showed in the liver. This tissue was subsequently treated in the same manner as in other experiments and 0.5 cm^3 was injected into each of 8 mice, 33 days of age. One mouse was killed each

day following injection. The results of this experiment are presented in figure 5. The low rates exhibited by the mice which were killed on the fifth and sixth days after injection are not easily explained. It seems possible, however, that the wave of mitosis in these particular animals either had already occurred or had not yet been initiated.

In this experiment 2 mice were saved to be killed as controls on the fourth and eighth days after injection. While no mitosis

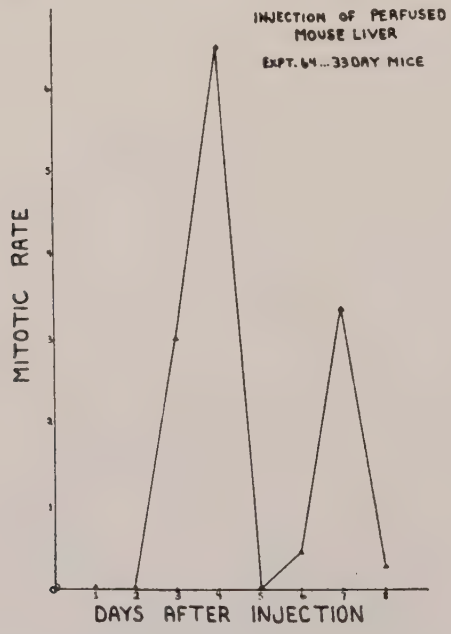

Figure 5

was found in the control animal killed on the day of injection and in the injected mice killed the first and second days after injection, the fourth day control exhibited a mitotic rate of 1.22 and the eighth day control, one of 2.72. The latter rate is considerably greater than that exhibited by the experimental animal killed on the eighth day after injection. We are at present at a loss to explain this but feel that it should be mentioned because we have found similar anomalous behavior in the controls in a number of other experiments.

Injection of autolyzed liver. The possibility that in the initiation of regeneration after partial hepatectomy the active agent is a specific product of autolysis of the tissue injured in the operation has been repeatedly suggested. Hence, experiments were conducted in which the liver tissue was first autolyzed for 4 days and then injected into the mice.

On the basis of the work of Gey [2] with tissue cultures the autolysis of mouse liver was carried out in a solution containing 500 units of penicillin [3] per cm^3 plus 40 mg % sulfanilamide made up in sterile Ringer's solution. Each adult liver to be autolyzed was ground up with 2 cm^3 of this solution, placed in a glass-stoppered weighing bottle and incubated at 37°C. for 4 days. Considerable experimentation in another connection has shown that smaller amounts of penicillin and sulfanilamide are adequate but the amounts used here give a considerable margin of safety. In no case has any putrefaction occurred and we have never lost an animal after injection of autolyzed tissue. At the end of the period of incubation it was necessary to grind the autolyzed mass in Ringer's fluid before it could be injected.

The results of experiments in which autolyzed liver was injected into young mice are presented in figures 6 and 7. Twenty-day-old sibling mice were used in experiment 34 (fig. 6); those killed on the second and seventh days after injection received 0.5 cm^3, the rest 0.2 cm^3. At the same time, 0.4 cm^3 was injected into each mouse of a litter 34 days of age. In experiment 39 (fig. 6) 28-day-old mice from 2 litters were given 0.5 cm^3 of the same material. In both of these experiments in spite of the preparatory autolysis the time taken for the effect to appear is the same as it would have been had fresh liver been used. This would indicate that the time which elapses before the effect appears is not to be attributed to the autolysis of the injected mass within the body cavity. It is also evident that the stimulating agent is not destroyed by the action of autolytic enzymes.

[2] Personal communication, Dr. G. O. Gey.

[3] The penicillin was supplied by Charles Pfizer & Co., Inc., Brooklyn, N. Y.

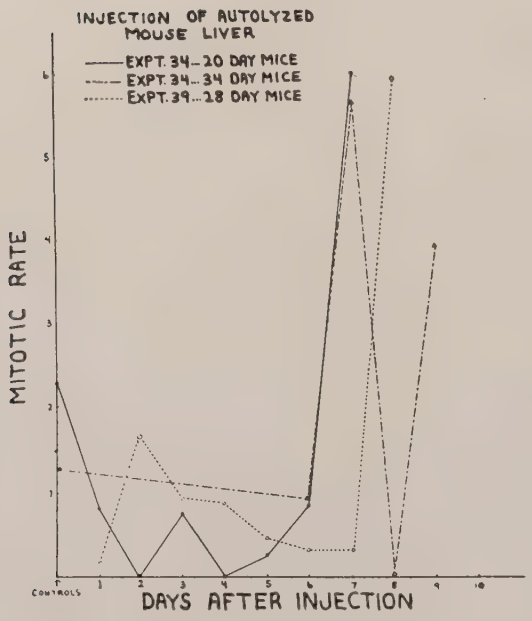

Figure 6

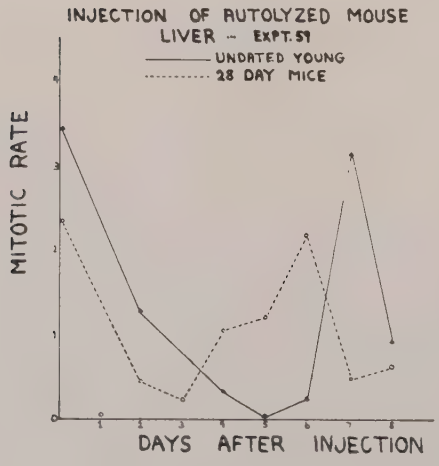

Figure 7

Figure 7 represents the results of an experiment in which sibling 24-day-old mice were given 0.5 cm³ and young animals of uncertain age but somewhat larger, 1 cm³ of autolyzed mouse liver. We publish these curves without comment as an example of an experiment the results of which are difficult to explain. The main points to be observed are that the mitotic rate at the start was high, the characteristic drop was slow, and the subsequent peaks of activity, while occurring at the right time, never go above the level of the initial controls.

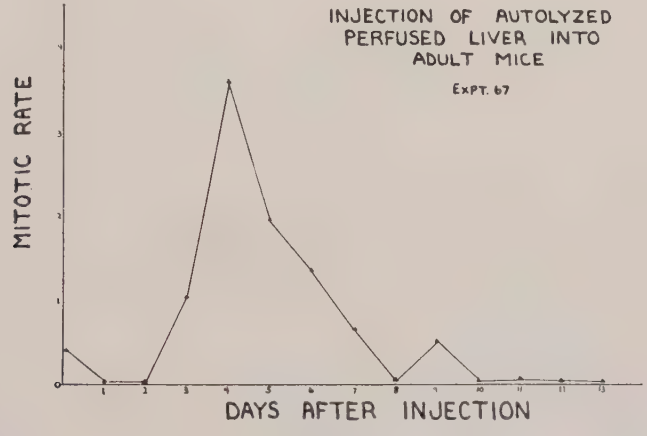

Figure 8

To eliminate any possible effects from blood an experiment comparable to experiment 64 above was performed. The liver was perfused before grinding, then autolyzed and 1 cm³ of the material was injected into each of 13 mice from 2 litters, one 8 weeks and one 9 weeks of age. The results are presented in figure 8.

Injection of mouse kidney. Mouse kidney was used in place of liver to discover whether the reaction is tissue specific. The 2 kidneys of each adult mouse donor were ground in 1 cm³ of Ringer's solution. In experiment 46 (fig. 9) 0.5 cm³ was injected into 35-day-old mice and in experiment 52 (fig. 9) 1 cm³ into 43-day-old mice. While the effect in these experiments is not as great as in those with liver, it appears to be

positive and would seem to indicate that at least part of the effect of the liver may be nonspecific.

Injection of guinea pig liver. Guinea pig liver was used in place of mouse liver to discover whether the effect is species specific. In order that the amounts should be comparable, about 1.5 gm of guinea pig liver was ground in 2 cm^3 of Ringer's fluid and injected in the usual manner.

In experiment 43 (fig. 10) 0.5 cm^3 of this material was injected into eight 29-day-old mice from 3 different litters, and in experiment 54 (fig. 11) 1 cm^3 each was given to young adults

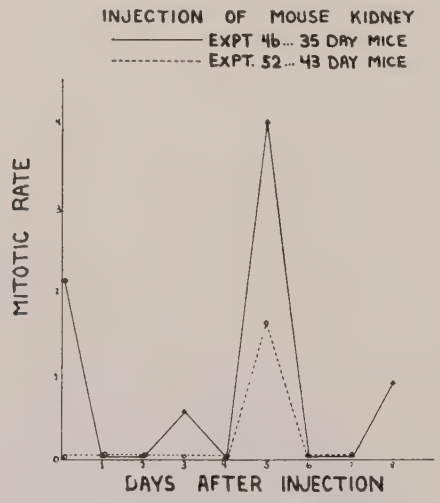

Figure 9

from 3 litters, 8, 10 and 12 weeks of age. In each of these experiments 1 animal showed a striking effect. In experiment 54, the fourth, fifth, sixth and tenth day animals show an amount of mitosis which we would consider significant in an experiment with adults.

In experiment 45 (fig. 12) 0.5 cm^3 was injected into sixteen 34-day-old mice from 2 different litters. The results from the 2 litters are plotted separately. The obvious absence of effect is puzzling though it may possibly be explained by assuming that the effect is of brief duration and not simultaneous in

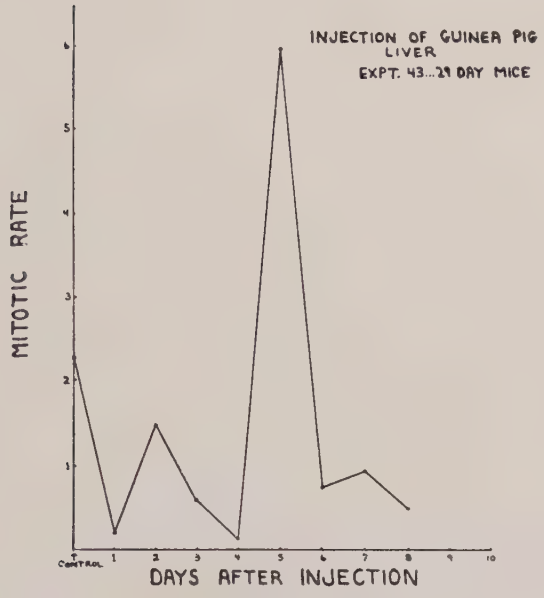

Figure 10

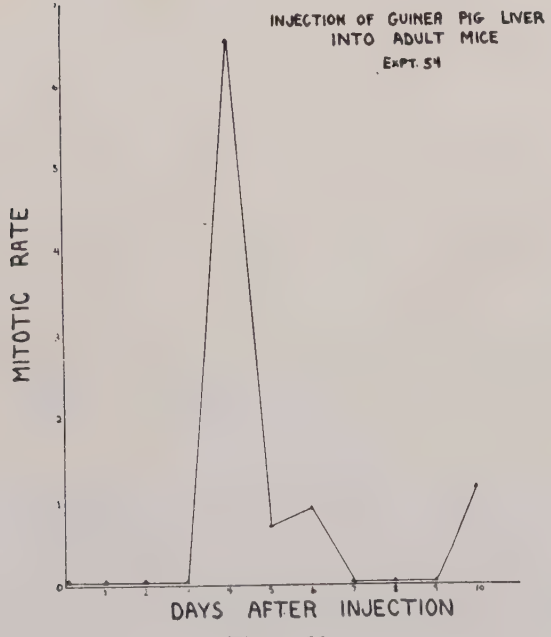

Figure 11

all animals so that none were killed in this experiment during a period of activity.

Injection of boiled egg yolk. Pfuhl ('38) induced mitosis in the liver of the guinea pig by the injection of trypan blue and attributed the effect to the particulate nature of the material. Hence we used boiled and ground egg yolk to obtain a particulate material of about the same consistency as the liver tissue but of an unorganized nature.

An egg was boiled for 20 minutes, allowed to cool for 10 minutes and the yolk removed aseptically. Two cm^3 of Ringer's fluid was added to each gram of yolk and the material was

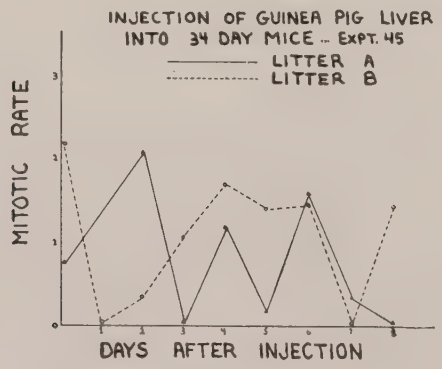

Figure 12

ground thoroughly. The resulting material was injected by means of a 23-gauge needle. In experiment 55 (fig. 13) 1 cm^3 of ground yolk was injected into sibling mice 23 days of age. In another experiment mice from 2 litters of 14-week-old adults were used, each injected mouse receiving 1 cm^3 of the ground yolk. The livers of the animals killed on the fifth, sixth and seventh days after injection contained a small amount of mitosis.

In both of these experiments a positive effect was obtained. Negative results would have indicated that it is not the particulate nature of the injected material which is responsible for the effect. From the positive results, however, the converse does not necessarily follow. Some less complex particulate

material must be tried. These experiments also imply that the stimulating agent is a thermostable one.

Injection of boiled mouse liver. Since positive effects were obtained with cooked egg yolk, we boiled mouse liver before injecting it in order to determine whether or not the active agent in liver tissue is thermostable. The entire liver from each adult mouse donor was ground in 1 cm³ of Ringer's fluid and then the test tube containing this material was immersed

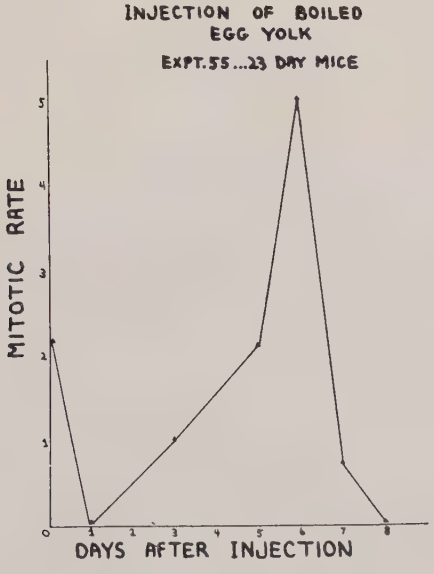

Figure 13

in a beaker of boiling water for 5 minutes. At the end of this period the cooked tissue was ground again in an additional 2 cm³ of Ringer's fluid and then injected. In experiment 63 (fig. 14) 10 mice, 29 days of age, from 2 litters each received 0.75 cm³ of the material and 1 was killed each day after injection. In another experiment in which adult mice, 16 weeks of age, from 2 litters received similar injections the animals which were killed on the third through the sixth days after injection showed a small amount of mitosis. From these ex-

periments we would conclude that the stimulating agent is thermostable since distinct effects comparable to those of uncooked liver have been obtained.

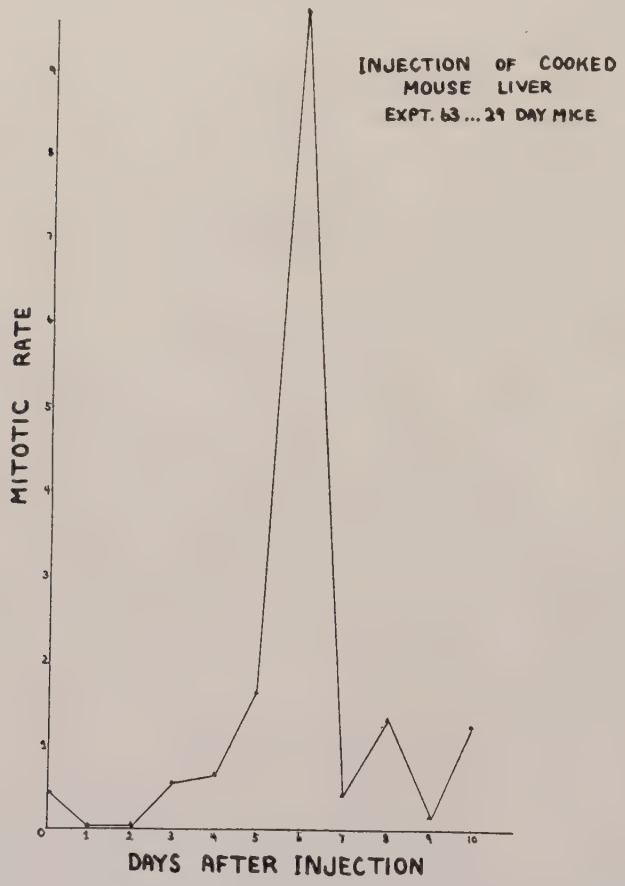

Figure 14

DISCUSSION

From these results and from the results of previous investigators who have found increased mitosis in liver cells following experimental procedures, it seems clear that the stimulation of mitosis in the liver is not a simple matter, and it would seem that the same mechanism or factors may not be

involved in each case. From the point of view of time of the effect, the experiments vary considerably. Marshak injected various products of liver cells related to chromatin, into a vein of hepatectomized rats and obtained increased mitosis after 3 hours. In simple hepatectomy, mitosis becomes very active in 2 or 3 days. Weiss and Wang obtained an increased mitotic index in the liver of host chick embryos on the third and fourth days after implanting liver onto the area vasculosa. Pfuhl, after injection of trypan blue into guinea pigs, found active mitosis on the sixth and eighth days only, none before or after. In our experiments, as in the similar ones of McJunkin and Breuhaus ('31), the peak comes from the fifth to the eighth day, and from the irregularity of the graphs it would seem that in an individual animal the activity might be of relatively short duration. The experiments of all these investigators differ in the agents employed and in the method of application.

Marshak's experiments differ from ours in that the material was injected intravenously and would therefore reach the liver immediately. In our experiments the fluid was rapidly absorbed from the injected material but much of the solid matter remained in the peritoneal cavity where it aggregated into masses. We have looked for but found no increased mitotic activity in the few hours immediately following the injection. It is possible that absorption of the effective factor of the injected material is slow. However, a colloidal material like trypan blue is absorbed from the peritoneal cavity into the blood stream with great rapidity and the timing of Pfuhl's effect using this as an agent is the same as ours. It would seem then that Marshak's results are due to something essentially different from ours.

The relationship of our results to the mitotic activity in liver regeneration is also problematical. The wave of activity in regeneration begins in the second and third day (Fishback, '29) at a time when in our experiments any mitosis that is already occurring seems to be temporarily suppressed. This suppression is indeed a problem in itself. It may be possible

that an inhibiting agent is included in the injected mass which has a transient but more energetic action than the stimulating one. Marshak reports an inhibitory effect of some of the materials he injected. On the other hand, there is a possibility that the suppression in the first few days is entirely secondary. It may, for example, be due to low food intake after the intraperitoneal injection of a large amount of material. In young animals in which there is still some mitosis in the liver this will be entirely suppressed after 24 hours without food, and furthermore, it does not begin again immediately after food is restored. This had been shown by Morpurgo working on young rabbits in 1889, and we have found it to be true in young mice. While our injected animals do not go without eating entirely, a reduced intake may be the explanation of the initial suppression of mitotic activity. If either of these explanations of the inhibition is correct, it is hard to see why it should not apply equally to regeneration.

It has been repeatedly suggested that mitosis in liver regeneration is due to some specific product of autolysis of injured liver tissue, e.g. by Schultz, Hall and Baker ('24). In the experiments of Weiss and Wang, since the implanted liver does not grow, it would seem that the effect is due to some product of autolysis of the liver. They called it a "specific organ discharge." McJunkin and Breuhaus suggest that there is given off from the injected material a "type-cell formative stimulant" which is of the nature of a food substance. We have tested autolyzed liver and found it as effective as fresh liver. That some such specific agent may be involved would seem probable from the facts that removal of liver causes regeneration of liver only and in Weiss and Wang's experiments only the liver seems to be affected. Although we have not made a critical survey, we have not found any other tissue affected in our experiments. On the other hand, we have obtained positive results with nonspecific agents. Both kidney and boiled egg yolk stimulate mitosis. Pfuhl's experiments also involved a nonspecific agent, trypan blue. However, until we know how these nonspecific agents produce their effects their bearing on

the question of tissue specificity is doubtful. Such agents as chloroform and phosphorous which produce liver injury may be considered to produce mitotic activity indirectly through specific agents liberated from the injured liver cells. While no injury is evident in most of our experiments, it would be difficult to rule this possibility out completely. Because of its great theoretical significance this question of a specific agent deserves further and more critical investigation.

Another possible explanation of these experimental results is that suggested by Pfuhl to account for the action of trypan blue in stimulating mitosis. His idea was that the trypan blue was taken up by the liver cell and clogged the machinery of secretion which was equivalent to the removal of some of the cells. The mitotic activity occurred as it does in compensatory hypertrophy or in regeneration, to make up the loss.

There are 2 ways that this concept could be applied to our experiments. First, the material injected is partially particulate, and colloidal material comparable to trypan blue is always present. Since it is colorless it would be difficult to demonstrate it in the liver cell, but it might still clog the machinery of secretion and be the cause of the mitotic activity. On the other hand, some of the materials injected might be eliminated through the liver cell in the bile as are the products of destruction of blood cells in the form of bile pigments. This might overload the liver and induce growth. With the possibility in mind that such material might be derived from the residual blood included in the injected liver, we perfused the liver in some experiments to eliminate it. This did not, however, alter the effect. We also used spleen in 1 experiment with the thought that as a seat of blood destruction it might contain materials which would overwork the liver. It did not produce any effect. On the basis of these preliminary experiments we would conclude that our experimental results are not due to the overworking of the liver in an attempt to eliminate materials injected. This would thus cast some doubt on the other possibility, i.e. that the material preoccupied the secretory mechanism because of its particulate nature. It

will require further work to rule out this possibility with finality.

To discuss more extensively the various suggestions as to the mechanism of stimulation of mitosis in the liver in the light of the experiments here reported would seem at present unprofitable. The hypothesis of a definite chemical agent, specific or nonspecific, would seem to be most easily tested in further experiments. It is obvious that in our successful experiments the material injected is chemically very complex. If the active agent is a definite chemical substance it must be thermostable up to 100°C. and resistant to the action of autolytic enzymes. It is evident that it could not be a native protein. A program is under way in which attempts will be made to isolate it by physicochemical methods or by differential centrifugation, or to identify it by injecting known materials derived from the liver.

We are at the same time working constantly on the problem of standardizing the mice themselves, for the variability is at times so great as to be disconcerting. The number of mitotic figures in animals used as controls is far from constant; animals of the same age and even from the same litter killed on the same day vary considerably. Occasionally an animal in the older groups has a large number of mitoses where none would be expected. These difficulties are so sporadic, however, that it would seem undesirable to attempt to smooth them out by large numbers and statistical treatment. It seems necessary to discover their origin and to eliminate them by experimental control. This we are now attempting to do by the use of animals of known genetic lines, by limiting litter size, and by the control of temperature and diet.

SUMMARY

1. The rate of mitosis in the livers of young mice is at first inhibited then stimulated by the intraperitoneal injection of pulped liver of the mouse and of the guinea pig. Boiled and autolyzed liver, pulped kidney, and boiled egg yolk were also effective. The inhibition lasts through the second or third day.

The increase reaches a peak from the fifth to the eighth day and then subsides. There is considerable variability in the results.

2. In similar experiments with adult mice in which there is no mitosis in the liver, a wave of mitosis of varying but usually small magnitude and duration occurs from the fifth to the eighth days.

3. These results are discussed in the light of the experience of other investigators who have obtained stimulation of mitosis in the liver, and of the explanations they have proposed. We have as yet no satisfactory explanation of the mechanism by which our results are produced.

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COMPARISON OF THE DISTRIBUTION OF AN HEXOSEDIPHOSPHATASE WITH GLYCEROPHOSPHATASE IN DIFFERENT TISSUES [1]

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SIX FIGURES

The presence of different types of alkaline phosphatases in tissues has for a long time been suggested. Folley and Kay ('36), in their comprehensive review, classified as separate phosphomonoesterases the enzymes hydrolyzing glycerophosphate, phytin (hexahydroxyhexahydrobenzene hexaphosphoric ester) and hexosediphosphate. Since then additional chemical evidence has been presented by Bodansky ('37), Bowers, Outhouse and Forbes ('40), Gomori ('43), Roche, de Laromiguiere and Laurens ('43) and Gould ('44). Nevertheless, the most recent critical opinion holds that the evidence for this differentiation of types of alkaline phosphomonoesterases is still not incontrovertible (Moog, '46).

The available information has been derived from macrochemical studies and includes knowledge of: (a) the substrate attacked, (b) pH activity curves, (c) response to activators and inhibitors, (d) the temperature of optimum activity, etc. Another source of information has been added by the introduction of a specific histochemical method for the localization

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of phosphatase (Gomori, '39; Takamatsu, '39). If it can be demonstrated that with the use of different substrates there is a difference in the histological and cytological localization of the precipitate which indicates enzymatic activity, it can be said that evidence corroborating the chemical indications of the individuality of the enzymes has been obtained. This method has been successfully used to differentiate alkaline from acid phosphatases.

Although our work has necessarily been temporarily interrupted, it should be of interest to record briefly the observations which have been made on the histochemical localization of an alkaline phosphatase utilizing the substrate fructose 1-6-diphosphate and the comparison of this localization with that of sodium β-glycerophosphatase.

MATERIALS AND METHODS

The tissues obtained from 5 species (mouse, rat, guinea pig, rabbit and man) included: esophagus, stomach, duodenum, colon, liver, pancreas, adrenal, kidney, bladder, epididymis, vas deferens, spleen, aorta, heart muscle, striated muscle, brain, lung and salivary gland. In addition, observations were made on a number of tumors: adenocarcinoma of the lung obtained at autopsy; epidermoid carcinoma of the larynx, papilloma of the larynx and reticulum cell sarcoma obtained as biopsies or after operation on man; spontaneous mammary tumor in Swiss mice; transplantable mammary carcinomas in C57 mice and dba mice and transplantable epidermoid carcinoma and adenocarcinoma of the intestine in mice of the A strain. The animal tissues were fixed immediately postmortem in chilled acetone. The human material could not be obtained immediately and was fixed after 1 to 6 hours. The details of the technique were similar to those given by Kabat and Furth ('41) with the exception that the precipitate was visualized with ammonium sulfide. In the fructose 1-6-diphosphate substrate [4] solutions, 80 mg per 100 cm^3 of solution was

[4] We are grateful for the sample kindly given us by Dr. H. Q. Woodard of the Memorial Hospital, New York City.

substituted in place of the Na β-glycerophosphate. In order to keep conditions as nearly uniform as possible, adjacent sections of tissue were used in the 2 substrates and controls, sections of comparable tissue from different species were mounted on the same slide, solutions were prepared at the same time and the slides were incubated and dehydrated at the same time. All tissues were incubated at 37°C. for 2½ hours, 12 hours and 70 hours; duodenum and kidney, which have large amounts of glycerophosphatase, were also incubated for 15, 30, 45, 60 and 90 minutes.

In preliminary experiments, the effect of pH was tested by incubating the sections for 2 hours in solutions buffered at 0.5 of a pH unit intervals from pH 3.5 to pH 10.5. In the acid range, lead salts were substituted for the calcium salts. No activity was observed in solutions more acid than pH 6.5. Starting at this point, a very weak reaction was observed which increased in intensity as the alkalinity increased. The maximum amount of precipitate was formed at pH 9.5, 9.7, and 9.9. This agreed well with the observations of Gomori ('43) on the activity of fresh tissue extracts toward hexosediphosphate. Therefore in the subsequent tests, the fructose 1-6-diphosphate solutions were maintained at pH 9.7 using a sodium barbital-sodium hydroxide buffer.

OBSERVATIONS

In general the observations on the localization of glycerophosphatase agreed with those which have already been made by a number of workers (Gomori, '39; Kabat and Furth, '41; Bourne, '43). With the long period of incubation, 70 hours, there were a few instances in which tissues previously described as being negative showed the presence of precipitate, or in which the amount of precipitate was greater than described. In guinea pig liver, the nuclei and nucleoli of the hepatic cells gave a faint reaction. The nuclei, cross-striations, myofibrillae, intercalated disks, capillaries as well as the surrounding connective tissue of cardiac muscle in the mouse, rat and guinea pig were heavily stained. Striated

muscle in all species also showed precipitate but much less than in cardiac muscle. The nucleus and cytoplasm of the acidophilic glandular cells of the mouse stomach gave a good reaction.

Comparison of tissues incubated with sodium β-glycerophosphate and fructose 1-6-diphosphate for 12 hours, 2½ hours or less showed the occurrence and distribution of precipitate, with a few exceptions, to be the same in both. Within the individual cells, the localization was also similar. For example, in the epithelial cells of the rabbit duodenum, the cuticular borders, supranuclear regions, nuclei and very faintly the cytoplasm showed the presence of precipitate after 30 minutes of incubation in either substrate (fig. 1, 2). The instances in which precipitate appeared only with one substrate have been summarized in table 1. It will be noted that they involve chiefly the nuclei, and that after 2½ hours incubation there is glycerophosphatase activity but no hexosediphosphatase, while the reverse is true after 12 hours incubation. This tendency for an increase in hexosediphosphatase activity with increase in length of incubation will be noted again.

Much more striking than the difference in localization after 12 hours or less of incubation was the difference in intensity of reaction. The tissues in nearly half the instances (summarized in table 2) could readily be distinguished because of the formation of a heavier precipitate after incubation with glycerophosphate. Only mouse liver and rat aorta, both of which show only a slight activity after 12 hours incubation gave a better reaction with fructose 1-6-diphosphate than with glycerophosphate. The remaining tissues appeared to be equally stained.

After 70 hours incubation, there was a change in the character of the results in that precipitate developed in some tissues which were negative after shorter incubation, and that a large number of tissues showed a greater hexosediphosphatase activity. Thus the esophagus of guinea pig and rabbit which showed less precipitate with fructose diphosphate than with glycerophosphate after shorter incubation, and the

esophagus of mouse, rat and human which had an equal amount, now showed a more intense reaction with fructose diphosphate. This was especially true in the rabbit. The results in the aorta of rat, guinea pig and rabbit were the most striking, as shown in figures 3–6. In all 3, the endothelial

TABLE 1

Summary of differences in localization of precipitate formed after various lengths of incubation with fructose 1-6-diphosphate (FDP) and with sodium β-glycerophosphate (GP).

SPECIES	ORGAN	TISSUE	REGION	FDP	GP
		2½ hours incubation			
Rat	pancreas	large duct epith.	nucleus	0	+
Rat	pancreas	islet	nucleus	0	±
Mouse	adrenal	blood vessel	all parts	0	++
Mouse	bladder	muscle	all parts	0	+
Rat	bladder	muscle	all parts	0	+
Guinea pig	bladder	muscle	all parts	0	+
Guinea pig	vas deferens	epithelium	nucleus	0	+
		12 hours incubation			
Guinea pig	liver	epithelium	nucleolus	+	0
Mouse	adrenal	capsule conn. tiss.	nucleus	+	0
Human	vas deferens	muscle	nucleus	+	0
Rat	aorta	intima	nucleus	+	0
Rat	aorta	media	nucleus	+	0
		70 hours incubation			
Rabbit	esophagus	muscle	all parts	+++	± or 0
Rat	epididymis	sub-epith. conn. tiss.	nucleus	0	++
Rat	aorta	endothelium	all parts	+++	± or 0
Rat	aorta	media	nucleus	+++	±
Rabbit	aorta	endothelium	all parts	+++	± or 0
Rabbit	aorta	media	nucleus	+++	± or 0

0 signifies no reaction; ±, very faint reaction; +, ++, +++, degrees of reaction.

lining and the nuclei of the components of the tunica media were definitely positive with hexosediphosphate but negative or almost so after incubation with glycerophosphate.

The observations on the liver agreed with the reports of Gomori ('41) and Bourne ('43) that the glycerophosphatase

activity is essentially negative. With the increase in incubation time, the nuclei and nucleoli of the hepatic cells showed a very faint reaction, but the duct cells and blood vessels remained essentially unstained. With fructose diphosphate, on the other hand, the nucleoli and nuclei were definitely positive, the cytoplasm was slightly positive and had a vacuolated appearance and the duct cells and blood vessels stained well. The intensity of reaction occurring in the other organs is summarized in table 2.

TABLE 2

Comparison of the intensity of phosphatase reaction after incubation with sodium β-glycerophosphate and fructose 1-6-diphosphate of various tissues from mouse (M), rat;(R), guinea pig (GP), rabbit (Rab.) and human (H).

	2½ OR 12 HRS. INCUBATION					70 HOURS INCUBATION				
TISSUE	M	R	GP	Rab.	H	M	R	GP	Rab.	H
Esophagus	0	0	+	+	0	—	—	—	—	—
Stomach	0	0	0	0	0	0	—	—	—	0
Duodenum	+	+	+	+	+					
Colon	+	0	0	+	0	0	—	—	—	—
Liver	—	+	0	0	+	—	+	—	0	0
Pancreas	+	0	+	0	0	+	0	0	—	0
Adrenal	0	+	0		+	0	—	0		0
Kidney	+	+	+	+	+					
Bladder	0	0	0	0	0	0	0	0	0	0
Epididymis	+	+	+			—	0	0		
Vas deferens	+		+		0	—	0	—		+
Spleen	0	0	0	0	0	0	0	0	0	0
Aorta	0	—	0	0	0	0	—	—	—	—
Heart muscle	0	0	0	0	0	0	0	0	—	—
Striated muscle	0	0	0	0	0	—	—	—	—	—
Brain	0	0	0	0	0					
Lung	0	0	0	0	0	0	0	0	0	0
Salivary gland	0	+	0	0						

+ signifies stronger glycerophosphatase reaction; 0, equal reaction; —, stronger fructose diphosphatase reaction; and blank spaces indicate that no tissues were incubated.

The tumors examined were incubated for 12 hours. All showed the presence of precipitate with both substrates but no difference other than a slightly darker glycerophosphatase reaction, in some instances, could be noted.

DISCUSSION

Using the histochemical method of Gomori ('39, '41), the presence of an enzyme capable of dephosphorylating fructose 1-6-diphosphate has been demonstrated in a variety of normal and neoplastic mammalian tissues. This raises the question, which has been posed before, of whether or not the enzyme which utilizes this substrate is an entity separate and distinct from the well known alkaline glycerophosphatase. Chemical evidence for the occurrence in certain tissues of a separate hexosediphosphatase has been presented by Kay (Folley and Kay, '36) and Gomori ('43). Recently Dempsey and Singer ('46) and Dempsey and Deane ('46) histochemically demonstrated a fructose diphosphatase in the rat thyroid and mouse duodenum respectively. The latter authors believe that a number of different phosphatases may exist in the duodenal mucosa, but no specific statements were made as to the individuality of a fructose diphosphatase.

The material presented in this paper indicates a slight difference in localization of precipitate formed with fructose diphosphate and with glycerophosphate; in activity as measured in terms of the amount of precipitate; and in pH optima. This may suggest the occurrence of a separate fructose diphosphate. However, special note should be made of the fact that many of the instances of difference in localization occurred only after an extremely long period of incubation. It is to be considered whether this long treatment could modify the structure of the cytoplasmic constituents so as to produce an artificial formation or distribution of precipitate. Incubation of tissue in buffer solutions alone for only short periods of time at 40°C. has been shown to affect the ribonucleic acid of cells (Stowell and Zorzoli, '47). Incubation in N/1 HCl at 60°C. also for only short periods of time diminishes the cytoplasmic basophilia of bacterial cells indicating some change in the components responsible for this affinity for basic dyes (Robinow, '45). On the other hand, the exact reproducibility of results and the occurrence of distinct histologic localizations within any given tissue give evidence

that the specificity of the reaction has not changed. Nevertheless, it is believed that the differences noted here were not sufficiently striking to warrant any final conclusions as to the occurrence of a separate fructose diphosphatase. Quite possibly a further study under diverse experimental conditions of the tissues showing differences may supply data which can better be used to corroborate the chemical evidence.

SUMMARY

1. A phosphatase utilizing the substrate fructose 1-6-diphosphate has been histochemically localized in a wide variety of normal tissues from mouse, rat, guinea pig, rabbit and human as well as in a few human and mouse neoplastic tissues.

2. Comparison with the alkaline glycerophosphatase activity in adjacent sections of tissue showed slight differences in the localization and in the activity as measured by the amount of precipitate formed in a given period of time.

3. No definite conclusions were drawn as to the identity of this phosphatase.

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PLATE 1

EXPLANATION OF FIGURES

1 Rabbit duodenum incubated for 30 minutes with Na β-glycerophosphate. $\times$ 330.

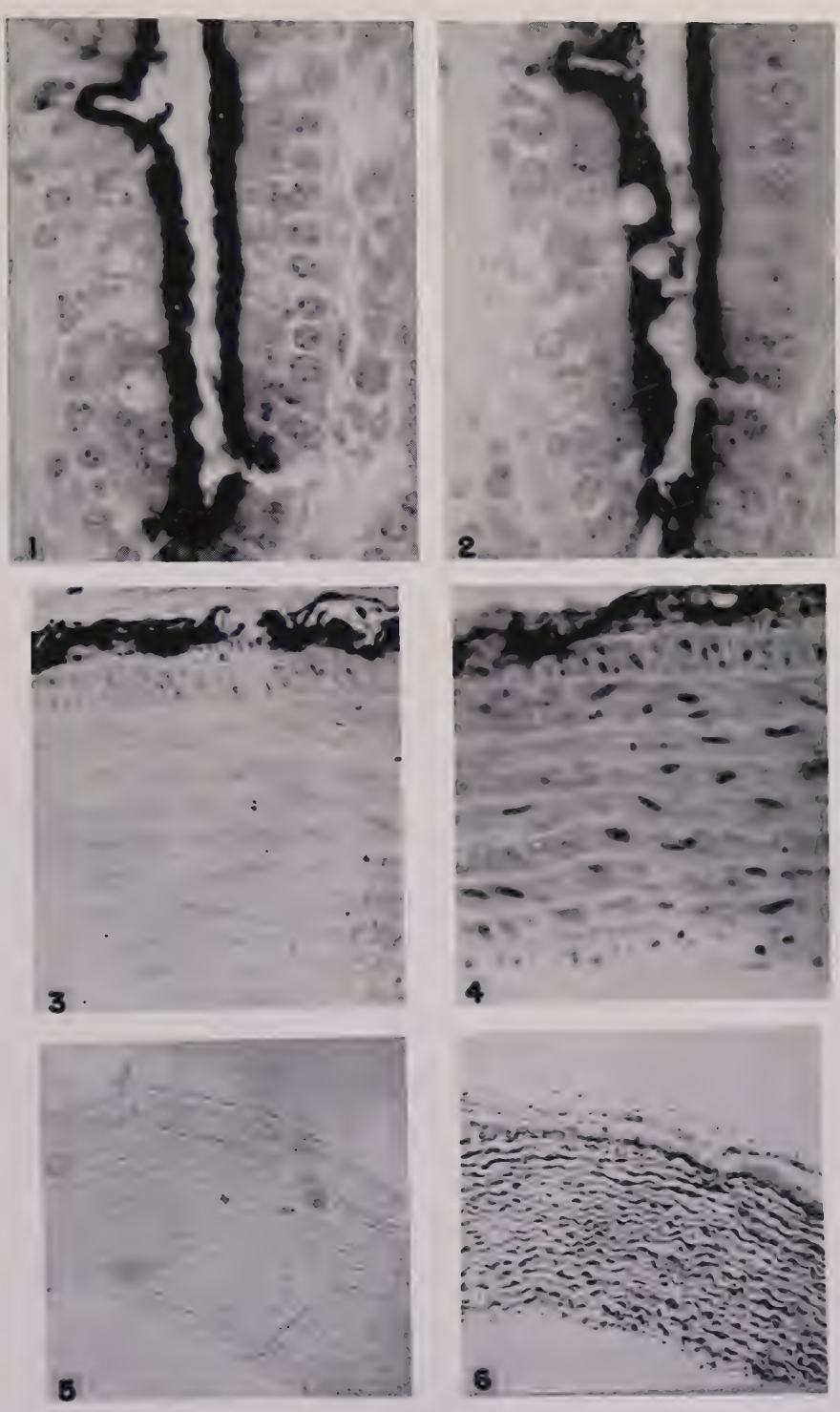
2 Rabbit duodenum incubated for 30 minutes with fructose 1-6-diphosphate. $\times$ 330.

3 Rat aorta incubated for 70 hours with Na β-glycerophosphate. $\times$ 330.

4 Rat aorta incubated for 70 hours with fructose 1-6-diphosphate. $\times$ 330.

5 Rabbit aorta incubated for 70 hours with Na β-glycerophosphate. $\times$ 75.

6 Rabbit aorta incubated for 70 hours with fructose 1-6-diphosphate. $\times$ 75.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EFFECTS OF GONAD REMOVAL ON THE ANTERIOR PITUITARY OF THE FOWL FROM 10 DAYS TO 6 YEARS

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ELEVEN FIGURES

This paper is one of a series on the cytology of the pituitary of the fowl and deals with the effects of castration and ovariectomy. It is well established in both birds and mammals that the pituitary is modified morphologically and physiologically by the removal of the gonads. The recorded results, however, are not uniform. Some of the differences are probably due to variations between species while others may be due to differences in observations. No attempt will be made to review the literature as this has been done in recent years by Severinghaus, '32, '37 and '39, who thinks the evidence is fairly conclusive that the basophiles increase in size and number and that the granular acidophiles recede following castration. At present we need to know, most of all, the facts, for without them correct interpretations cannot be derived. What are the facts in the fowl?

My observations, limited to White and Brown Leghorn varieties, cover an age range beginning with 10 days and ending with 6 years. They give us, I think, the significant changes during this period.

The gonads were removed in all White Leghorn fowls at ages varying from 36 hours to 14 days. The exact age at castration of the Brown Leghorn capons is unknown. They were received from L. V. Domm, of the University of Chicago, and

[1] Contribution no. 362.

I wish to express my appreciation for this cooperation. I am indebted to W. R. Breneman of our own laboratories for the White Leghorn material.

THE MALE

For orientation purposes let me remind the reader that at the time of hatching there are no functional basophiles in the fowl pituitary, but granular acidophiles are numerous in the posterior part of the gland. At 10 days, however, large functional basophiles are present in the mid juxtaneural region and a few are scattered throughout the posterior end. Acidophiles are still prominent. From 10 days to sexual maturity, the basophiles increase in number and there is some growth in size (Payne, '42). During this period the acidophiles also increase in number but relative to the basophiles they are fewer. At the time of sexual maturity the basophiles are the dominant cell type.

When castrated at 36 to 48 hours there are no visible effects on the pituitary at 10 days, but changes are present at 15 days, are more marked at 20 days, and become even more pronounced in later stages. There are 4 of these initial changes, which clearly distinguish the castrate pituitary from controls, (1) the increase in number and enlargement of the basophiles, (2) the increase in size of the nucleoli in the basophiles, (3) the degranulation and recession of the acidophiles, and (4) the increase in size of the pituitary.

With the exceptions of the size of the nucleoli and the size of the cells, basophiles of castrates are not much different from those in controls. The cytoplasm, following methyl green and acid fuchsin, stains medium to light green, but not dark. It is usually uniform in texture, sometimes granular or flocculent but whether the granules are comparable to the secretory granules of acidophiles seems doubtful. The Golgi material is similar in controls and castrates and while it is difficult to be certain my impression is that the mitochondria are more numerous in castrates. All appearances indicate that these cells are not mere storehouses but that they are

releasing secretory materials. Breneman's assays (unpublished data) of castrate pituitaries point in the same direction. In agreement with earlier work on the rat he finds the total amount of FSH present at any given age is greater in the castrate pituitary than in controls. Per unit of weight, however, they are similar. This latter finding seems somewhat contrary to the cellular picture because the number of large basophiles in castrates is greater than in controls and they are distributed throughout the entire gland while in controls this is never the case except at the time of sexual maturity. The apparent discrepancy can be explained, however, if the small basophiles of controls also contain stored secretions and if the large basophiles of castrates are releasing.

There are no signet ring cells.

The nucleoli which are conspicuous in controls become much larger in castrate basophiles and are usually sperical (figs. 1, 2 and 3). Occasionally 2 or 3 nucleoli may be found within a single nucleus. The rapidity of the castrate pituitary changes as well as the degree, depend much upon external conditions. When the environment is unfavorable or when food is reduced, castrate changes may be delayed indefinitely. When these conditions are at an optimum, the major changes previously mentioned are practically complete by 35 days of age. Beyond 35 days the pituitary grows in size and the increase is due primarily to further growth and multiplication of the basophiles. At what age the castrate pituitary reaches its maximum size is uncertain but judging by the material at hand, the maximum is attained at age 223 days and possibly earlier. This conclusion is reached by comparing areas of mid-saggital sections. Breneman ('44) gives the weight increase of the pituitary from 5 to 90 days for control White Leghorn fowls, the increase being from 0.9 mg to 8.3 mg. Recently (unpublished) he has supplemented these data to include ages up to 105 days and has in addition studied the weights of castrate pituitaries from 20 to 105 days. At 20 and 105 days the control pituitaries weighed respectively 2.1 and 9.5 mg. The castrate pituitary increased in weight from 2.8

mg at 20 days to 14.6 mg at 105 days. The castrate pituitary weights then average about 1/3 greater than in controls.

The White Leghorn male is sexually mature at 90 days plus or minus. At what age control pituitaries reach their maximum size and maximum degree of efficiency in FSH output I am uncertain, but from a study of the areas of mid-saggital sections I find that some pituitaries at 90 days reach a size which is not exceeded at any time in older birds.

I have described certain modifications in the pituitaries of control fowls as aging changes. At least the modifications appear with increased age and become more pronounced as age progresses. There are 3 conspicous changes. The mitochondria of the basophiles become vesicular, fuse and increase in size. Acini appear first in the posterior end of the pituitary and later in other regions. Pigment forms in the secretory cells but not until they have passed beyond their period of activity. (For more complete descriptions of these changes see Payne, '42 and '46.)

These same transformations, but varying in degree and rate, occur in castrate pituitaries. Acini are fewer in number than in controls, and pigment develops to a lesser degree. The mitochondrial changes, however, are more marked and may appear as early as 56 days in contrast with about 65 days in controls. This time difference, however, may be of little significance as there is much variability in both castrates and controls. The principal difference is that the mitochondrial changes in castrates progress more rapidly than in controls. A capon of 1 year 5½ months of age may have as many basophiles affected as cocks of 5 to 9 years but the mitochondrial vesicles are not as large. Beyond 1 year 5½ months the mitochondrial vesicles continue to fuse and the number of basophiles affected increases until at 4 years all such changes are more marked than in the older cocks. Changes similar to those in the basophiles are now found in many of the chromophobes at the periphery of the pituitary. At 6 years of age the capon pituitary (Brown Leghorn) is much more modified. Cells are gone in large areas (fig. 8) and their places taken

by mitochondrial vesicles varying in size but ranging up to the size of large basophiles. See figure 4 for a series of stages taken from a single field showing transitions from granular mitochondria to large vesicles. Figures 1 to 7 and 9 to 11 are drawn to the same scale and show various stages of vesicle formation. Even though a high percentage of basophiles exhibits mitochondrial changes to a greater or lesser degree, there are a few such cells which, from all appearances, are probably still functional. In controls, basophiles become inactive and degenerate, not only through mitochondrial changes but also through recession in size and through pycnotic changes in the cytoplasm and nuclei. Basophiles affected by this second kind of change are more numerous in controls than in castrates. A few small granular acidophiles remain in all castrate pituitaries studied. The small body described in acidophiles of old cocks (Payne, '42) is not present in old castrates. Regeneration of small fragments of testicular tissue has little or no effect on pituitary changes following castration.

FEMALES

I have described (Payne '46) similarities and differences between the development and behavior of the secretory cells in pituitaries of control male and female fowl. At the time of sexual maturity the pituitaries are much alike but previous to that time the basophiles are on the average more abundant in the male and the acidophiles more numerous in the female. With respect to aging changes as exhibited by mitochondria in the basophiles the two sexes are strikingly different. The changes begin as early as 65 days in the male and gradually increase with age, while in the female no such changes are present at 8 years of age. They are present, however, at 13 years, but as I have no material between 8 and 13 years I do not know the exact time of their first appearance.

Are the pituitary changes following ovariectomy similar to or different from those following castration in the male? When the left ovary is removed at 2 days the basophiles are enlarged and the number increased at 30 days. Recession of the acido-

philes, involving degranulation and shrinkage in size, has already begun. The changes are similar to those described in castrate male pituitaries, but they are not as marked. The difference, however, may be due to the fact that the basophiles of the female pituitary are about 5 days delayed in their initial development when compared to the male. Beyond 30 days, pituitaries of ovariectomized hens are similar to those of castrate cocks when all gonad tissue has been removed. Even the aging changes within the basophiles, judging by pituitaries from 30 days to 2⅓ years of age, are now similar to those found following castration. Apparently the presence of the testes on the one hand and of the ovaries on the other with their accompanying secretions account for the differences found in pituitaries of controls. When the gonads are removed the cause of the differences no longer exists.

From my studies of pituitaries of gonadectomized male and female White and Brown Leghorn fowl certain conclusions seem clearly established. (1) The basophiles enlarge and increase in number. (2) The acidophiles degranulate and regress. (3) The differences in aging changes found in the mitochondria of the basophiles of control male and female White Leghorn fowl disappear. (4) Aging changes as observed in the mitochrondria of the basophiles are more pronounced in gonadectomized fowl than in controls.

Do such facts along with others in any way help in an understanding of the activities and functions of the secretory cells of the pituitary? It is generally assumed that the basophiles secrete the gonadotropic hormones and various suggestions have been made as to the functions of the acidophiles. These suggestions include the production of a growth hormone, prolactin, the thyrotropic and adrenotropic hormones and others. Without attempting to draw conclusions I wish to point out certain relationships between the basophiles and acidophiles which are found in the fowl.

1. Granular acidophiles are present during late embryonic development and at the time of hatching, that is, before the appearance of basophiles and before the gonads begin to

function, judging by the effects of gonad secretion upon comb growth. Of course interstitial cells, comparatively speaking, are more numerous than in later stages and what part they play, if any, remains unknown.

2. Basophiles are not present before 5 to 10 days post hatching.

3. Acidophiles are numerous and basophiles undeveloped in fowls reared under adverse environmental conditions or fed on a limited diet.

4. Acidophiles are numerous and basophiles undeveloped in dwarf chicks.

5. Acidophiles are numerous and basophiles undeveloped in female Cornish fowl of 6 months of age (males not examined). Cornish are larger and grow more rapidly than White Leghorns and reach sexual maturity much later.

6. As far as our experience goes acidophiles are more numerous and basophiles less developed in chicks injected with estrogens.

7. Acidophiles decrease and basophiles increase following castration and ovariectomy.

8. Generally speaking the acidophiles are more numerous and basophiles less so in female fowl than in males, except possibly during the active laying period when the female pituitary approaches that of the cock.

Looking at the facts derived from these many sources it is obvious that acidophiles are most numerous when basophiles are absent or few in number and that the acidophiles are fewer when the basophiles are well developed. In other words there seems never to be a high development of both acidophiles and basophiles in the same pituitary. Could this mean that the basophilic secretions, either directly or indirectly, influence adversely the development of the acidophiles? I do not have the answer but the facts justify the question, at least.

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PLATE 1

EXPLANATION OF FIGURES

1 Three large basophiles from capons. Note the cells and nucleoli are larger than in figures 2 and 3, basophiles from cocks and hens respectively.

2 Basophiles from cocks.

3 Basophiles from hens.

4 A series of stages, drawn from a single microscopic field, in the transition of granular mitochondria through small to large vesicles. The largest vesicle is larger than the basophiles in figures 1, 2 and 3.

5 A typical basophile showing granular mitochondria.

6 A basophile in which all mitochondria appear as vesicles. This is unusual.

7 Another exception is a cell filled with mitochondria in which some show fusion but as yet no vesicle formation.

All figures in this plate are drawn to the same scale.

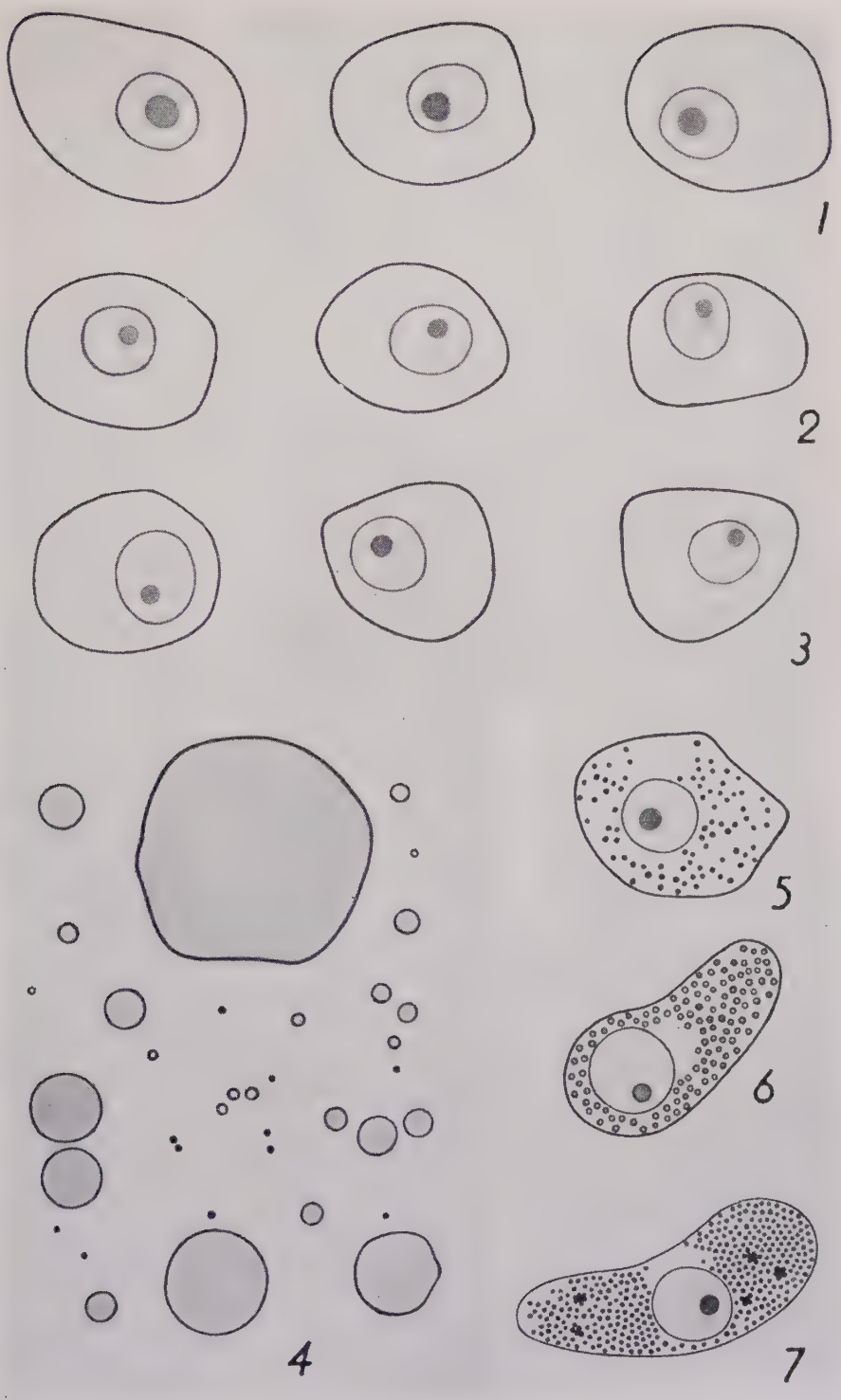
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PLATE 2

EXPLANATION OF FIGURES

8 A part of a section through a 6 year old castrate pituitary. Practically all cells are gone and the vesicles occupy the areas formerly filled with cells.

9, 10 and 11 Three drawings of castrate basophiles showing different stages of vesicle formation and fusion. Drawn to the same scale as figures 1 to 7.

8
9
10
11

THE INFLUENCE OF MATERNAL HORMONES ON THE REPRODUCTIVE ORGANS OF SUCKLING RATS [1]

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THIRTEEN FIGURES

INTRODUCTION

It has been found that the prostate glands of male and female rats can be stimulated by hormones from the testes, the ovaries and the adrenal glands. Transplantation experiments have demonstrated that male prostate tissue can be maintained in a normal functional-appearing histological condition in virgin female hosts but that such grafts are markedly stimulated when the host females are pregnant or lactating. Seminal vesicle tissue does not respond to hormones that are circulating in virgin female hosts but grafts recovered from females that have bred repeatedly and have lactated continually during the experiment may be stimulated histologically (Price, '37, '41). The female prostate gland which occurs spontaneously in some female rats is normally in a somewhat regressed histological state in adult virgin females (Price, '39) but when prostates are found in pregnant and lactating females the glands frequently appear well developed histologically (Burrill and Greene, '42).

These findings make clear that the hormones that are circulating in the pregnant and lactating rat stimulate growth and differentiation in male and female prostate tissue and to some extent in seminal vesicle tissue. This suggests that if

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such hormones reach fetal or suckling young the growth and the development of the male and female accessory reproductive organs may normally be influenced to some degree, perhaps at critical periods, by maternal hormones. There is abundant evidence that hormones traverse the placenta, at least at certain periods in pregnancy, and under normal and experimental conditions affect the accessory reproductive organs of the male and female fetus (see Courrier, '45). It has been shown by many workers that milk contains hormones in both normal and experimental conditions and that suckling young are affected by hormones administered to lactating mothers. The degree to which the suckling young may normally be influenced by maternal hormones has not, however, been studied.

Ovariectomy of the lactating female rat offers a logical approach to the problem. If maternal hormones go through the milk to the young, removal of maternal ovarian androgen might produce reduction in the weight or a change in the histology of the prostate and seminal vesicles of suckling young; removal of maternal ovarian estrogen might reduce the weight of the uterus (which is sensitive to estrogen in the very young female) or inhibit uterine differentiation. If maternal ovarian hormones reach the suckling young in effective amounts there is also the possibility that there might be inhibition of the gonadotrophic hormone of the pituitary of the young rat. Spaying of the mother might thus remove an inhibitory hormone influence and result in an increase in gonad weight in the young.

The present work is based upon ovariectomy of lactating females and a detailed study of the reproductive tracts of the suckling young.

MATERIAL AND METHODS

Two strains of rats were used in these experiments, one the female prostate strain which has been inbred in this laboratory and has an incidence of approximately 80% of spontaneously occurring prostate glands in the females and the

other, our laboratory stock (designated as M stock in tables 2 and 3) which has an incidence of less than 2% of such glands. The reactions of the 2 strains were compared to see whether a difference in response could be observed. The female prostate strain had the added advantage that not only could the prostate of the young suckling female be studied but also that of the lactating mother in which the prostate histology then served as an indicator of androgenic hormone action in the normal and spayed mothers. The data for such a study are given in table 1.

The experimental procedure consisted of spaying lactating females, thus removing the source of maternal ovarian hormones, and comparing the weight and histology of the reproductive organs of their suckling young with the organs of suckling young of non-spayed mothers. Spaying was performed 2 days after parturition when lactation was well established. The operation did not interfere with lactation for there were no significant differences in body weight between the young of spayed and non-spayed mothers except in normal males at 20 days of age. To determine the effect of the removal of all possible sources of gonad-secreted hormone some of the suckling males and females of spayed and normal mothers were gonadectomized at 2 days of age. This procedure was followed in only the female prostate stock.

It was evident that if there was any influence from maternal hormones on the organ growth of the suckling young it should be apparent within the first 2 weeks of life when the young were entirely dependent upon their mother's milk. Therefore the majority of the litters were autopsied at 8 or 14 days of age but a few from the female prostate stock were allowed to live until 20 or 26 days for special study of the prostate gland.

A total of about 1000 rats was used in the experiment: approximately 100 normal or spayed lactating mothers and their litters which included 100 young castrated males, 100 young spayed females, and 700 young normal males and females.

At autopsy, the body weights of the suckling rats were obtained and the gonads, ventral prostate glands, seminal vesicles and uteri were rapidly dissected, weighed on a torsion

balance which was accurate to 0.2 mg and fixed in Bouin's fluid. Normal mothers that were autopsied with their litter were weighed, the ovaries, uteri and female prostate glands (when present) were dissected and weighed and the uteri and the female prostate glands were fixed for histological study. Spayed mothers were weighed, the uteri were examined, a search was made for any possible ovarian fragments and the female prostate glands were weighed and fixed. Table 2 summarizes the weight comparisons at 8, 14, and 20 days of age for all the organs that were studied except the female prostate.

Serial sections of the organs of the suckling young, and of the prostates of the mother were made at a thickness of 10 μ and stained in Harris or Ehrlich's hematoxylin. In the histological study the diameters of prostate acini, testis tubules and ovarian follicles were measured and compared in the young of spayed and non-spayed mothers. The organs of approximately 200 rats were studied histologically.

A statistical analysis was made of body and organ weights to determine whether any significant changes occurred as the result of treatment. Body weights and weights for each organ in all rats similarly treated at any one age in each stock were grouped and the mean weights were computed. Comparisons were made between the means of comparable groups with the mothers normal and the mothers spayed using the "t" test (Fisher's formula). Significant differences were found in some organs but since the differences in mean weight were relatively small further statistical treatment was advisable and rigorous analyses of variance and co-variance were made for all data at each age level.[2] A part of this analysis for body weights and for some of the organs is presented in table 3.

[2] The more involved analyses are in complete agreement with the simpler series of "t" values given in table 3. These values were obtained by applying the standard "t" test separately to each pair of comparable groups. It is vital to consider the litter means as the basis for calculating group means and variances. The close resemblance between littermates as contrasted with the larger differences between animals of different litters (same type of mother) invalidates the "t" test if the standard error of the difference between the group means is based on individuals without regard to the division into litters.

EXPERIMENTAL RESULTS

Lactating females

The female prostate gland is in a functional histological state in some lactating females presumably because of stimulation from ovarian or adrenal androgens or both. It was of interest to determine whether the removal of the ovaries produced detectable changes in the mother's prostate gland and thus indicated a decrease in the androgens which might be available to the suckling young. The results of such a study of the prostate gland of spayed and non-spayed lactating females at various ages after parturition are summarized in table 1. The glands were classified histologically as (1) "functional," containing acini with high epithelium and cell light areas or (2) "distended," possessing moderately or greatly distended acini but with a low castrate type of epithelium, or (3) "regressed," with small acini, low epithelium and a large amount of inter-acinous connective tissue. The histological state of the female prostate frequently varies greatly in different parts of the same gland and in the 2 lobes. The prostates were classified on the basis of the best histological development that was found in the gland.

As is shown in table 1, prostates containing at least some acini with high epithelium and cell light areas were found at 8, 14 and 20 days of lactation in normal females although the proportion dropped from 5 of 6 glands at 8 days to 2 of 5 at 20 days of lactation. Figure 1 illustrates a portion of a "functional" female prostate from a normal female at 8 days of lactation and shows high epithelium and cell light areas. When the mothers were spayed 2 days after parturition only 1 functional-appearing prostate was found at any period of lactation. In the spayed females at 8 days of lactation 4 of the 8 glands that were examined contained distended acini with low epithelium as shown in figure 2, and 4 glands were in a regressed and atrophic state. A larger proportion of completely regressed glands was found at all ages in spayed lactating females than in non-spayed lactating females. Atrophic

prostates have been found in virgin, pregnant and lactating females but their occurrence is infrequent.

While the histological condition of the female prostate varies widely under the same conditions in different animals

TABLE 1

The female prostate gland in lactating rats.

DAY OF LACTATION	NO. CASES	* WEIGHT mg		HISTOLOGY		
				Functional light areas	Distended acini	Regressed
Normal ♀♀						
		5.5	7.6			
8	6	7.6	R3.2	5	1	0
		12.4	3.6			
		R1.2	R2.2			
14	6	R1.5	R3.4	2	1	3
		3.6	6.8			
		2.6	3.6			
20	5	3.9	6.8	2	1	2
		3.8				
30	1	7.0		0	1	0
Spayed ♀♀[1]						
		4.2	R1.8			
8	8	4.0	3.9	0	4	4
		4.8	R1.8			
		3.6	R2.0			
		5.0	R1.8			
		R2.2	R1.8			
14	11	3.8	4.4	1	2	8
		5.6	R0.8			
		R0.6	R1.2			
			R2.0			
		3.6	2.4			
20	5	1.6	4.1	0	1	4
		2.5				
		2.2				
26	3	4.6		0	1	2
		2.2				

* R — only right lobe present.

[1] All females spayed 2 days after parturition.

and even in different parts of the same gland, a comparison of the prostates in spayed and non-spayed lactating females shows that the ovaries of lactating females produce hormones which stimulate the female prostate. The possibility that the adrenal cortex may also be producing androgenic substances has not been eliminated. In fact, the gland that was found in a functional histological state in a spayed lactating female 14 days after parturition, 12 days after spaying, suggests that there was probably in that animal an additional source of stimulation other than the ovaries, or that there was an exceptionally slow prostate regression after ovarian removal. However, for the purpose of this experiment it may be concluded that removing the ovaries decreases the amount of androgen circulating in the lactating female rat and might thus reduce the amount present in the milk.

The number of cases of female prostate glands in non-spayed females at the various lactation periods is too few to permit definite conclusions to be drawn but the indications are that the titer of androgenic hormones, as judged by the histological condition of the prostate, is high just after parturition and decreases during lactation. This corroborates the findings of Burrill and Greene ('42).

The weights of the prostates given in table 1 demonstrate the great variability of the gland which made it impossible to determine whether spaying produced a loss in weight. In general the lightest glands (2 mg or less) were atrophic and the heaviest contained some acini with high epithelium and light areas in the cells or distended acini that were not greatly regressed. However, weight was not always correlated with histological state.

Suckling young

Eight days of age. At this age the accessory reproductive organs of the normal young male rat are small and undeveloped. The prostate gland (fig. 3) contains small solid acini with lumina present in the ducts and beginning to develop

TABLE 2

Summary of the organ weights of suckling rats of normal and spayed mothers.[1]

ORGANS OF SUCKLING RATS	8 DAYS OF AGE			14 DAYS OF AGE			20 DAYS OF AGE		
	No. rats	Mean wt. mg	Diff.	No. rats	Mean wt. mg	Diff.	No. rats	Mean wt. mg	Diff.
♂♂ Prostate									
Mother normal	38	1.75		31	4.37		18	10.88	
Mother spayed	32	1.65	.10	29	3.43	.94	16	10.04	.84
♂♂ Prostate (M stock)									
Mother normal	48	1.71		46	3.85				
Mother spayed	41	1.61	.10	45	3.22	.63			
♂♂ [2] Prostate									
Mother normal	21	1.11		21	1.65		10	2.70	
Mother spayed	25	.93	.18	22	1.52	.13	10	2.37	.33
♂♂ Seminal vesicle									
Mother normal	38	1.66		31	3.94		18	4.76	
Mother spayed	32	1.65	.01	29	3.35	.59	16	4.46	.30
♂♂ Seminal vesicle (M stock)									
Mother normal	48	1.87		46	3.88				
Mother spayed	41	1.79	.08	45	3.50	.38			
♂♂ [2] Seminal vesicle									
Mother normal	21	.61		21	1.42		10	1.85	
Mother spayed	25	.52	.08	22	1.04	.38	10	1.99	—.14
♂♂ Testis									
Mother normal	38	22.87		31	66.11		18	141.36	
Mother spayed	32	22.96	—.09	29	63.01	3.1	16	127.23	14.13
♂♂ Testis (M stock)									
Mother normal	48	22.10		46	59.92				
Mother spaled	41	23.60	—1.5	45	61.70	—1.78			
♀♀ Uterus									
Mother normal	28	5.95		23	14.06		13	19.73	
Mother spayed	27	5.70	.24	21	13.68	.38	10	18.64	1.09
♀♀ Uterus (M stock)									
Mother normal	44	5.23		32	13.09				
Mother spayed	40	5.42	—.19	35	13.19	—.10			
♀♀ [2] Uterus									
Mother normal	25	5.76		20	10.46		8	15.07	
Mother spayed	27	5.43	.33	21	10.81	—.35	7	15.52	—.45
♀♀ Ovary									
Mother normal	28	1.39		23	3.39		13	10.70	
Mother spayed	27	1.37	.02	21	3.54	—.15	10	9.17	1.53
♀♀ Ovary (M stock)									
Mother normal	44	1.28		32	2.53				
Mother spayed	40	1.36	—.08	35	2.53	0			

[1] Mothers were spayed 2 days after parturition; young rats were gonadectomized at 2 days of age.

[2] Castrated ♂♂ or spayed ♀♀.

in the proximal portions of the acini. The seminal vesicles have a simple central lumen with small side branches. The testis tubules are solid and have a germinal epithelium that is 1 to 2 cells in thickness. The female prostate consists of very small solid acini (fig. 5). The uterus of the female is small, the lumen is slit-shaped and there are no glands. The ovary has many small follicles and in some follicles the beginning of antrum formation is evident.

No histological changes could be found in any organ in the suckling young as a result of the spaying of the mother. The prostate gland of a normal suckling male (fig. 3) with a normal mother resembles exactly the glands from young males of spayed mothers. The prostate of a young castrated male (fig. 4) with a normal mother is indistinguishable from the glands of castrated males with spayed mothers. If any hormones are reaching the suckling young through the milk they are not increasing the size of the prostate acini. The factor which is of the greatest importance in prostate development is male hormone from the testes of the suckling young as can be seen by comparing figure 3, the prostate of a normal male, with figure 4, the prostate of a castrated littermate. The great reduction in size of the acini in figure 4 and the absence of lumina are evident although mitoses are still relatively abundant.

In the weight comparisons in table 2 a small decrease in weight is shown in the prostates of young normal and castrated males in the groups in which the mother was spayed. These differences are not statistically significant. However, a far more pronounced difference in weight was found as the result of castration. In the female prostate stock the mean weight of the young normal male prostate when the mother was not spayed was 1.75 mg and when the mother was spayed it was 1.65 mg; castration of the young reduced the glands to 1.11 mg in the normal mother group and 0.93 mg in the spayed mother group. These differences between the weights of the normal and castrate prostate are highly significant and reflect the inhibited growth and development that was found histo-

logically after castration. The normal prostate gland in figure 3 weighed 1.6 mg as compared with 1.0 mg for the prostate of the castrated littermate in figure 4.

The seminal vesicle showed the same hormone relationships as the prostate gland and was not affected histologically or in weight in normal males or castrated males when the mother was spayed. Castration of the young male produced, histologically, inhibition of development of the branches from the lumen so that the gland had a simple structure, and the mean weight was markedly and significantly reduced.

The testes of the young males were not affected histologically by the spaying of the mother nor was there any significant change in weight. However, in the M stock there was an increase in mean testis weight from 22.10 mg to 23.60 mg when the mother was spayed and this difference of 1.5 mg had a P value of 0.06, approaching significance. This suggests that there may be a physiological difference between the 2 strains but more data would be necessary before the point could be definitely established. If such a difference was found to exist it might indicate a high level of estrogen in adult females of the M (non-prostate) strain immediately after parturition.

Removal of the mother's ovaries had no detectable effect upon the prostate histology in the suckling females. The prostate in the normal suckling female is very small and undeveloped (fig. 5) and resembles that of the castrated male (fig. 4). The large acinus on the left of figure 5 is the duct of the lobe; the remaining acini are very small and are solid. The gland was too small to be weighed.

Spaying the suckling female itself did not change the histological appearance of the prostate and in this the female prostate differed from that of the male. The female gland, however, is in such an undeveloped condition in the normal female that it is doubtful whether any inhibition could be recognized, especially since it was impossible to obtain weights.

The uteri and ovaries of suckling females were not changed histologically nor in weight when the mother was spayed. The slight differences given in table 2 were not significant.

In addition, removal of the ovaries of the young females did not affect the uteri histologically nor were the weight reductions which occurred (5.95 mg to 5.76 mg in the group with the normal mothers) statistically significant.

Fourteen days of age. In the normal male the prostate has developed markedly since the 8-day-stage (compare figs. 3 and 6) and has more than doubled in weight (table 2). The acini are much larger than at 8 days, most of them have a lumen, the epithelium is high and it contains cell light areas. The seminal vesicles possess a more complicated structure and have more than doubled their 8-day weight. The testis tubules are larger in diameter than at 8 days but they are still solid. Testis weight has almost tripled.

The prostate gland in the normal female has increased in size, the acini are larger, and small lumina are beginning to form (compare figs. 5 and 12). The uterus has developed histologically to the point where glands have formed and have penetrated to the base of the endometrium (fig. 8). Uterine weight has more than doubled.

When the mothers were spayed there were no consistent changes in the prostate histology of the normal suckling males. Figures 6 and 7 show respectively the prostate glands of a normal male with an unspayed mother and a normal male with a spayed mother, and in acinous size, epithelial height and cell light areas the 2 glands are quite similar. The result of spaying the mother was apparent only in the weights, for the gland in figure 6 weighed 4.2 mg and that in figure 7 weighed 2.9 mg. The data presented in table 3 show that with no significant differences in body weight between the 2 groups (young with normal mothers and with spayed mothers), the mean prostate weight of normal males in the female prostate stock was 4.37 mg when the mothers were normal and 3.43 mg when the mothers were spayed. This difference of 0.94 mg had a P value of <0.01 and was highly significant. In the M stock the weights were 3.85 mg and 3.22 mg for the same groups and the P value was 0.02, again significant. The hormone coming through the milk from the

TABLE 3

The effect of ovariectomy of the mother on the organ weights of suckling rats. 14 days of age.

	NO. RATS	NO. LITTERS	$\bar{m}$ and $\sigma \bar{m}$ [1]	D AND σ D [2]	t	P
♂♂ Body wt.						
Mother normal	31	13	22.72 ± .78	1.50 ± 1.13	1.3	not sig.
Mother spayed	29	13	21.22 ± .82			
♂♂ Body wt. (M stock)						
Mother normal	46	11	19.67 ± 1.01	—.11 ± 1.41	—.07	not sig.
Mother spayed	45	11	19.78 ± .97			
♂♂ [4] Body wt.						
Mother normal	21	11	21.60 ± .81	.98 ± 1.39	.71	not sig.
Mother spayed	22	11	20.62 ± 1.16			
♂♂ Prostate						
Mother normal	31	13	4.37 ± .13	.94 ± .23	4.0	<.01
Mother spayed	29	13	3.43 ± .19			
♂♂ Prostate [3] (M stock)						
Mother normal	46	11	3.85 ± .21	.63 ± .25	2.5	.02
Mother spayed	45	11	3.22 ± .13			
♂♂ [4] Prostate						
Mother normal	21	11	1.65 ± .08	.13 ± .13	1.0	not sig.
Mother spayed	22	11	1.52 ± .10			
♂♂ Seminal vesicle						
Mother normal	31	13	3.94 ± .07	.59 ± .17	3.5	<.01
Mother spayed	29	13	3.35 ± .15			
♂♂ Seminal vesicle [3] (M stock)						
Mother normal	46	11	3.88 ± .82	.38 ± .12	3.2	<.01
Mother spayed	45	11	3.50 ± .86			
♂♂ [4] Seminal vesicle						
Mother normal	21	11	1.42 ± .05	.38 ± .11	3.5	<.01
Mother spayed	22	11	1.04 ± .09			

[1] $\bar{m}$ — group mean weight (average of litter means), body weights in grams, organ weights in milligrams; $\sigma \bar{m}$ — standard error of the group mean.

[2] D — difference between group means; σ D — standard error of the difference.

[3] means and variances adjusted for body weight.

[4] Castrated ♂♂.

mother was evidently effective in promoting growth of the prostate gland. Maternal hormone was not necessary for histological differentiation which was dependent upon male hormone secreted by the testes of the suckling males. Castration produced great inhibition in development of the prostate as compared with the normal (see figs. 6 and 9). The diameters of the acini have increased since the 8-day-stage in figure 4 but lumina are only beginning to appear. Ovariectomy of the mother did not result in any further inhibition of histological state although the acini in figure 11, the prostate of a castrated male with a spayed mother appears somewhat smaller than the acini shown in figure 9, a castrated male with a normal mother. This was apparent in some cases but it was not a consistent finding and it is undoubtedly to be attributed to the variability which is marked in the glands of the castrates.

Castration of the suckling males caused marked inhibition of prostate growth as is evident in the weights in tables 2 and 3. The mean weight of the gland in normal males with normal mothers was 4.37 mg and in the castrated littermates 1.65 mg, a difference which was highly significant. When the mother was spayed the mean weight in normal males was 3.43 mg and in castrated littermates 1.52 mg. A comparison of the mean weights of the glands of castrates in the normal-mother, spayed-mother groups revealed a slight reduction of 1.65 mg to 1.52 mg which was not significant (table 3). In view of the significant changes in the prostate weights of normal males which resulted when the mothers were spayed, this finding in castrated males was unexpected, especially since the results reported below for seminal vesicles of the same castrated males showed a significant reduction in seminal vesicle weight in the spayed-mother group.

The seminal vesicles of the normal suckling males were not noticeably influenced histologically by spaying of the mother but, as with the prostate gland in the same normal males, highly significant differences in weight were found between the glands in the groups with mothers normal and mothers

spayed. In the female prostate stock the mean weight reduction with spaying of the mother was from 3.94 mg to 3.35 mg and in the M stock from 3.88 mg to 3.50 mg. Both differences had a P value of <0.01 (table 3).

In the young castrated males there was great inhibition of the histological development of the seminal vesicle so that the glands were much simpler in structure than in the normal. The weight changes as compared with normal littermates were from 3.94 mg to 1.42 mg in the normal-mother group, and from 3.35 mg to 1.04 mg in the spayed-mother group. The comparison between the weights of the glands of the castrates in the 2 groups (1.42 mg and 1.04 mg) demonstrated a highly significant decrease in weight when the mothers were spayed (P value of <0.01). These findings show that hormone from the mother's ovaries reaches the seminal vesicles of the suckling young, presumably via the milk, and stimulates growth as reflected in increased weight.

Testis histology and weight were not influenced by removal of the mother's ovaries. There was a slight increase in weight (table 2) in the M stock with spaying of the mother but it did not approach significance as the difference in testis weight in that stock had done at 8 days of age.

The female prostate gland of the suckling rats was not changed histologically by spaying the mother. The prostate in a normal female with a normal mother is illustrated in figure 12, and while growth and enlargement of the acini have progressed and the beginning of lumina formation has taken place since 8 days of age (fig. 5) the gland is still in a very undeveloped state comparable to that of the castrated male (figs. 9 and 11). Ovariectomy of the suckling female produced only slight changes in the prostate. These consisted of somewhat smaller acini and less developed lumina but there was little inhibition (fig. 13).

The data for the weight comparisons in the female prostate were not entirely satisfactory. Only glands with bilateral lobes were used for weight comparisons since unilateral right or left lobes are usually, although not always, lighter in weight

than the combined bilateral lobes. In consequence, while 17 female prostate glands were found in the 23 normal suckling females of the normal-mother group, only 6 were bilateral and the numbers of cases in all groups were small. The numbers that could be compared were 6 prostates in normal females with mothers normal (mean weight 0.65 mg); 11 prostates in normal females with spayed mothers (mean weight 0.61 mg); 11 prostates in spayed females with normal mothers (mean weight 0.46 mg); 14 prostates in spayed females with spayed mothers (mean weight 0.51 mg).

Spaying of the mother did not significantly affect the weight of the prostate gland in normal suckling females nor in spayed females. Spaying of the suckling female apparently reduced the weight (0.65 mg to 0.46 mg in the mother-normal group; 0.61 mg to 0.51 mg in the mother-spayed group) but these differences were not statistically significant. As reported above, there were indications that the removal of the ovaries of the suckling young caused slight histological changes in the female prostate.

There were no apparent effects upon uterine histology or weights in the suckling females when the mothers were spayed. Removal of the ovaries of the young females produced no inhibition of differentiation but a marked and highly significant weight decrease was found (14.06 mg to 10.46 mg with normal mothers; 13.68 mg to 10.81 mg with spayed mothers). Uterine glands appeared as well developed in spayed females (fig. 10) as in the normal littermates (fig. 8). The uteri shown in figures 8 and 10 were from females with the mother spayed which shows that the development of uterine glands is completely independent of ovarian hormones from any source. The size difference is apparent in the 2 organs (the weight of the uterus in fig. 8 was 15.4 mg and that in fig. 10, 10.8 mg) but the degree of development is similar.

The ovary in young females was not affected in any way by spaying of the mother.

Twenty days of age. Between 14 and 20 days of age the prostate gland of the young male continues rapid growth and

histological differentiation. The seminal vesicles grow more slowly than the prostate (table 2) and increase in complexity of structure but the epithelium remains low and undifferentiated. The testes increase markedly in weight, the tubules enlarge and lumina develop in many of them.

The female prostate grows rapidly, the acini increase in diameter, the epithelium becomes relatively high and light areas indicative of a "functional" state appear in the cells. The development of the gland varies greatly in different individuals but the prostates of all 11 normal females examined at this age had some acini with high epithelium and light areas. The uterus grows relatively slowly and continues to differentiate. The ovary grows rapidly and the follicles increase markedly in size.

A significant decrease in body weight was found in the normal males at this age when the mothers were spayed. Since this was not found in any group at any other age it did not represent an interference with lactation.

The prostate and seminal vesicles in normal suckling males were not changed histologically when the mother was spayed and the weight differences (slight reductions) were not significant in spite of the significant difference in body weight. The testes, however, were significantly reduced in the young males in the mother-spayed group undoubtedly as a result of the reduced body weight since the coefficient of correlation between body weight and testis weight was 0.97 at 20 days of age.

The prostate glands of castrated males had developed markedly since the 14-day-stage shown in figures 9 and 11. There was no difference that was attributable to spaying of the mother, and the glands of both experimental groups had some acini that were distended and had high epithelium and typical light areas. The histological condition was variable but 4 of 5 castrate prostates in the mother-normal group and 7 of 8 in the mother-spayed group contained at least some high epithelium with light areas. The differentiation which occurs

between 14 and 20 days in the prostate of the castrated male is not dependent upon hormone stimulation from the mother.

The prostate glands of the castrates were far below normal weight (table 2) but the small additional reduction in weight found in young of the mother-spayed group was not significant.

Removal of the mother's ovaries did not produce any detectable effects upon the seminal vesicle histology or weight in castrated males. The glands of castrates were very simple in histological structure and had advanced little if any beyond the 14-day stage.

Ovariectomy of the mother did not change the histology or weights of the female prostate, uterus, or ovaries in the young females. Ovariectomy of the suckling female did inhibit the histological development of the female prostate to some degree but the gland had developed remarkably from the 14-day stage (fig. 13) and cell light areas were found in the 5 glands examined. Weights of bilateral lobes were too few for statistical analysis but a loss in weight was indicated with spaying. There was no evidence that hormone from the mother assists in the differentiation or growth of the female prostate in the 14- to 20-day period.

Twenty-six days of age. Only a relatively few rats were used at this age and all the results were negative; no influence of the mother on any organ was detected.

DISCUSSION

It has been known for many years that milk contains hormones under normal conditions. Brouha and Simonnet ('27) tested extracts of human milk, taken during the first 9 days of lactation, on adult spayed or immature mice and found that estrus was induced (indicating the presence of female hormone). Aschheim and Zondek ('28) mentioned the finding of small amounts of female hormone in human milk. Brühl ('29) found female hormone in untreated human milk on the third and seventh days post partum and anterior pituitary hormone in 1 case on the third day. Frank, Goldberger and Gustavson (quoted by Frank, '29, p. 119) demonstrated large

quantities of female hormone in the milk of goats in estrus. Yaida and his collaborators ('29) reported that estrus occurred in spayed rats and mice when they were injected with extracts of the milk of pregnant cows. Estrogen was present in the milk early in pregnancy, increased in amount as pregnancy progressed and dropped in titer after parturition so that no female hormone could be detected after the fifth day.

Heim ('31a and b; '32) obtained positive Zondek-Aschheim tests with human colostrum and milk (centrifuged) to 5 days post partum but after that day the findings were negative. Winter ('32) found anterior pituitary hormone in human colostrum from the seventh month of pregnancy to 6 days after parturition. He did not detect female hormone in colostrum. Konsuloff ('34) administered untreated human colostrum to immature mice and caused ovarian and uterine changes indicating the presence of anterior pituitary hormone. He compared the hormone content of colostrum and urine from the same woman and found that, in general, the colostrum was far more active in enlarging the uteri of test mice than was the urine but that the amounts of hormone in colostrum were more variable than in urine. He did not obtain positive Zondek-Aschheim tests with colostrum or milk after parturition and this was also true of cow and sheep colostrum. In addition to the finding of anterior pituitary hormone in human colostrum, Konsuloff demonstrated a hormone in sheep colostrum before parturition that produced contractions in the excised uterus of a guinea pig in the second half of pregnancy. This "pregnancy contraction curve" was similar to the one obtained in the guinea pig uterus with the urine of pregnant women. Lacassagne and Nyka ('34) injected mice with human colostrum (sterilized) and induced a characteristic estrous reaction. They concluded that colostrum contains large amounts of female hormone. No reports have been found in the literature of any attempts to test milk for the presence of hormone with androgenic activity.

Hormone has been found not only in the colostrum and milk of adult females (women, cows, sheep and goats) but also

in the "witches' milk" of new-born children. Joseph ('29) injected mice with "witches' milk" from children 4 and 5 days after birth and induced estrus but at 7 days the test was negative. Winter ('32) was unable to detect female hormone or anterior pituitary hormone in "witches' milk" but he stated that he had very small quantities of the substance and could use only a few test animals.

Some experimental studies have shown that milk of lactating females that were treated with estrogen contained detectable amounts of the substance. Hain ('35) injected the milk of estrogen-treated rats into spayed rats and determined that there was female hormone in the milk (rather less than 5 R.U. per cm^3). Lawson, Stroud and Williams ('44) found small quantities of female hormone in extracts of milk of heifers that had been implanted with tablets of synthetic estrogens. Their assay method was the subcutaneous injection or intravaginal application of the milk extracts in ovariectomized rats and the study of vaginal smears. In these experiments the acetone extract of the milk of the untreated control cow induced a positive vaginal response in 4 of 5 test animals. This was interpreted as apparently a case of contamination of the milk with estrogen in the laboratory.

Treatment of lactating female rats and guinea pigs with estrogen has resulted in profound effects upon the reproductive tracts of the suckling young (Courrier, '30; Hain, '35 and '36; Weichert and Kerrigan, '42) which have been interpreted as estrogen effects via the milk. Especially interesting in regard to the effectiveness of hormone in milk is the work of Lawson, Stroud and Williams ('44) who administered cow's milk to which had been added known quantities of estrogen, to ovariectomized rats in place of drinking water or as a diet. Positive vaginal responses were obtained with a total stilboestrol intake in milk used as drinking water (5-day period) of 1.9 to 2.3 μg. When a whole milk diet was fed, a total intake of as low as 1.3 μg in a 5-day period gave a positive vaginal reaction.

These studies prove that hormones (estrogenic and anterior pituitary) have been found in colostrum and milk under normal conditions of lactation; that estrogens can be recovered in the milk of estrogen-treated mothers; that the reproductive tracts of suckling young guinea pigs and rats have been profoundly affected by female hormone administered to lactating mothers and that ovariectomized rats have been brought into estrus by drinking cow's milk to which synthetic estrogens have been added. The question then arises whether hormones from the mother reach the suckling young in effective amounts under normal conditions and whether maternal hormones can play any role in the normal growth and development of the reproductive tract of the young.

The experiments reported in the present work were devised to test whether hormones from the maternal ovaries normally influence in any way the reproductive system of suckling rats. By spaying the mother, the source of maternal ovarian estrogen, progesterone and/or ovarian androgen was removed. Studies made on the mouse and rat give some indication of the amounts of these hormones circulating in lactating females. Atkinson and Leathem ('46) studied the morphology of the mouse uterine endometrium using the method of Hooker ('45). They concluded that at parturition there is a moderate estrogen level; within 2 days estrogenic stimulation disappears and is not apparent again during the lactation period. Progestin increases during the first 4 days after parturition and reaches a peak on the fifth day; a moderate to high level is maintained until the twelfth day when the titer begins to fall, reaching the initial low level by the twenty-first day.

Burrill and Greene ('42) reported for the lactating rat that, judged by the histological condition of the female prostate gland, there was an effective level of androgen for the first 15 days of lactation but that for the next 6 days there was an absence of androgen or a subthreshold level. They did not attempt to identify the androgen or determine the source. The study reported here of the histological condition of the prostate glands of lactating female rats showed that removal

of the ovaries lowers the titer of ovarian androgen and would thus reduce the amount available for the suckling young.

If the mouse studies can be applied to the rat the estrogen level would be expected to fall rapidly in the lactating female within a few days following parturition and the progesterone level would be expected to rise and be maintained for about 2 weeks. From research on the rat it has been established that ovarian androgen from whatever source is high for 2 weeks of lactation and is lower or absent thereafter. If these normally circulating hormones reach the suckling rat it seems probable that ovarian androgens rather than estrogens might be effective agents, and the removal of ovarian androgen might produce detectable changes in the suckling young.

The criteria used in the suckling male for the detection of maternal ovarian androgen action were the growth of the prostate and seminal vesicles and the differentiation of the prostate. It was recognized that testis-secreted hormone controls the early growth of both of these glands to a very large extent and that while differentiation of the prostate occurs in the absence of the testes the seminal vesicles remain undeveloped (Price, '36). It has been shown that the prostate of the young castrated rat differentiates under the influence of adrenal androgens (Howard, '37, '38, '41; Burrill and Greene, '39a, '40).

In the suckling female the growth and differentiation of the female prostate were studied in relation to maternal ovarian androgen. Growth of the young female prostate is under the control of ovarian androgen from the ovaries of the suckling females to some extent but differentiation of the gland takes place in the absence of the ovaries. This differentiation is attributable to adrenal androgen (Burrill and Greene, '39b, '41).

The findings in this study of the weights of the accessory reproductive organs support the contention that maternal androgens do reach the suckling young and that a part of the growth of the normal male prostate and seminal vesicle between birth and 14 days of age is attributable to androgenic

stimulation from the mother. A part of the growth of the seminal vesicles of castrated males is dependent on maternal ovarian androgens but the prostate gland of these same castrated males is not significantly reduced in weight when the mother is spayed. This fact suggests that the prostate gland in the castrated male with a spayed mother is responding to some other androgenic influence which compensates for the loss of the maternal ovarian androgen. The rat prostate is capable of reacting to adrenal androgens while the seminal vesicle is not. This leads to the speculation that spaying of the mother might increase the titer of maternal adrenal androgen which, if it reached the suckling male, would stimulate the prostate.

The prostate gland of suckling females was so unsatisfactory for weight purposes that it was impossible to determine whether maternal androgens were contributing to its growth. It was found, however, in earlier work (Price, '41, '42) that the male prostate was more responsive than the female prostate to ovarian and adrenal androgens.

None of the glands was influenced in cellular differentiation by maternal hormones. The rapid differentiation of the prostate of the castrated male and the prostate of normal and spayed females in the age period between 14 and 20 days of age was definitely independent of any hormones from the mother's ovaries.

The weight and the histological development of the uterus of the suckling female were used in this study as a test for the presence of effective amounts of maternal female hormone. It has been demonstrated that the uterus of the very young rat is sensitive to estrogen. The administration of 0.166 μg daily (total dose 0.996 μg) of estradiol benzoate to suckling females from birth to 6 days of age enlarged the uterus (Price and Ortiz, '44) and even 0.02075 μg daily (total dose 0.166 μg) from birth to 8 days of age was very effective (Price and Harvey, unpublished). The growth of the uterus, however, is not under the control of female hormone from the ovaries of the suckling female between birth and 8 days of age as judged by the uterine weights in young normal and spayed

females. Neither is any of this early growth or later growth dependent upon female hormone from the mother's ovaries because spaying of the mother did not cause significant weight changes in the uteri of suckling females at any age. The uterine growth between 8 and 14 days of age is partly under the control of the ovaries of the suckling female, but the differentiation of uterine glands which appear at about 10 days is independent of female hormone influence from the animal's own ovaries and from those of the mother. No ovarian hormone is necessary for the development of uterine glands in the rat (see figs. 8 and 10). This is in contrast to the situation in the opossum for which Moore and Failor ('44) reported that bilateral ovariectomy of the pouch young "almost completely inhibits" gland formation. In the rat it must be concluded that, as judged by weight and differentiation, the uterus of the suckling female is not influenced by maternal estrogen. If ovarian female hormone from the mother reaches the suckling young the titer is too low to affect the uterus in any detectable way.

A study by Marx ('45) indicates that maternal estrogen may be reaching the suckling rat in effective amounts as judged by another criterion. She found that lactation could be induced in suckling female rats by the injection of testosterone propionate. After the rats were weaned similar hormone treatment resulted in the development of thick-walled alveoli but no lactation occurred. She concluded that since lactation requires 3 hormones (1) estrogen (2) progesterone, corticosterone or testosterone and (3) pituitary lactogenic hormone, that one or both of the other necessary hormones were supplied by the mother's milk.

Myers ('19) reported that a slight secretion appeared in the milk ducts of newborn male and female rats 12 hours after birth. It was more abundant by 4 or 5 days of age and decreased in amount after the second week. This suggests that under normal conditions all the hormones necessary for mammary secretion (perhaps all from the mother) are available to the suckling young in minimal amounts. The addition of

testosterone propionate in the experiments of Marx increased the reaction and thus produced true lactation.

The withdrawal of maternal estrogen through the milk has been postulated by Weichert ('42) to explain the delayed implantation and prolonged term of pregnancy in pregnant lactating rats that are suckling litters. He suggested that estrogen which was essential for the preparation of the endometrium for implantation was removed in the milk. He supplied small amounts of estrogen and obtained implantation at the normal time in 68% of the animals, a finding which supported his theory.

In the present research, in which it was found that maternal hormone affects the accessory reproductive organs of suckling males there is little doubt that the effect was mediated via the milk. While the mother was undoubtedly excreting hormone in the urine and feces there is no reason to believe that rats under 2 weeks of age would be affected by hormone from these sources.

The significance of the findings that maternal hormone stimulates growth in the reproductive tract of the suckling young rat lies in the fact that it proves that suckling animals have access to hormones from sources other than their own endocrine glands. These experiments in the rat dealt only with maternal ovarian hormone but the possibility exists that maternal adrenal and anterior pituitary hormones also reach the young by way of the milk. It is probable that the rat is not unique in its response to maternal hormone. It also seems likely that the less developed the reproductive tract of the young is at birth the greater the effect from the mother might be. It remains to be demonstrated whether maternal hormones play a significant role in the development of the reproductive tract of the suckling young.

SUMMARY

The removal of the ovaries of lactating female rats 2 days after parturition produces:

1. Histological changes in the female prostate gland of the lactating females, indicating a reduction in the level of ovarian androgen.

2. Weight reductions in the prostate and seminal vesicles of the suckling males as compared with suckling males of non-spayed mothers. The weight changes were very small at 8 days of age and were not statistically significant but at 14 days of age the weight differences were highly significant (P values of <0.01 to 0.02). By 20 days of age there was no effect upon the prostate and seminal vesicles of the young when the mother was spayed.

3. No effect upon the histological differentiation of the male prostate and the seminal vesicles and no effect upon the weight or histology of the testes, ovaries, female prostate or uterus of the suckling young.

CONCLUSIONS

Ovarian androgens of lactating female rats reach the suckling young in effective amounts via the milk and contribute in some degree to the growth of the male prostate and the seminal vesicles. There is no evidence from this study that estrogens from the mother have any effect upon the organs of suckling male or female rats.

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EXPLANATION OF PLATES

All photomicrographs were taken at a uniform magnification (× 150). All young rats that were gonadectomized were operated at 2 days of age; all mothers that were spayed were operated 2 days after parturition.

PLATE 1

EXPLANATION OF FIGURES

1 Prostate gland of a normal lactating female 8 days after parturition. The epithelium in the acini is moderately high and cell light areas are present in some parts of the epithelium. Weight, right lobe 3.2 mg.

2 Prostate gland of a spayed lactating female 8 days after parturition. The epithelium is low and castrate in type and no light areas appear. Weight, bilateral lobes, 4.8 mg.

3 Prostate gland of an 8-day-old normal male (mother normal). Lumina are beginning to form in the proximal portions of the acini. Weight 1.6 mg.

4 Prostate gland of an 8-day-old castrate male (mother normal). Littermate to the male in figure 3. The acini are small and without lumina. Weight 1.0 mg.

5 Prostate gland of an 8-day-old normal female (mother normal). The acini are small and solid. The large structure on the left is the duct.

6 Prostate gland of a 14-day-old normal male (mother normal). The epithelium is high and cell light areas are evident. Weight 4.2 mg.

7 Prostate gland of a 14-day-old normal male (mother spayed). The histological condition is like that of the gland in figure 6 but the weight was only 2.9 mg.

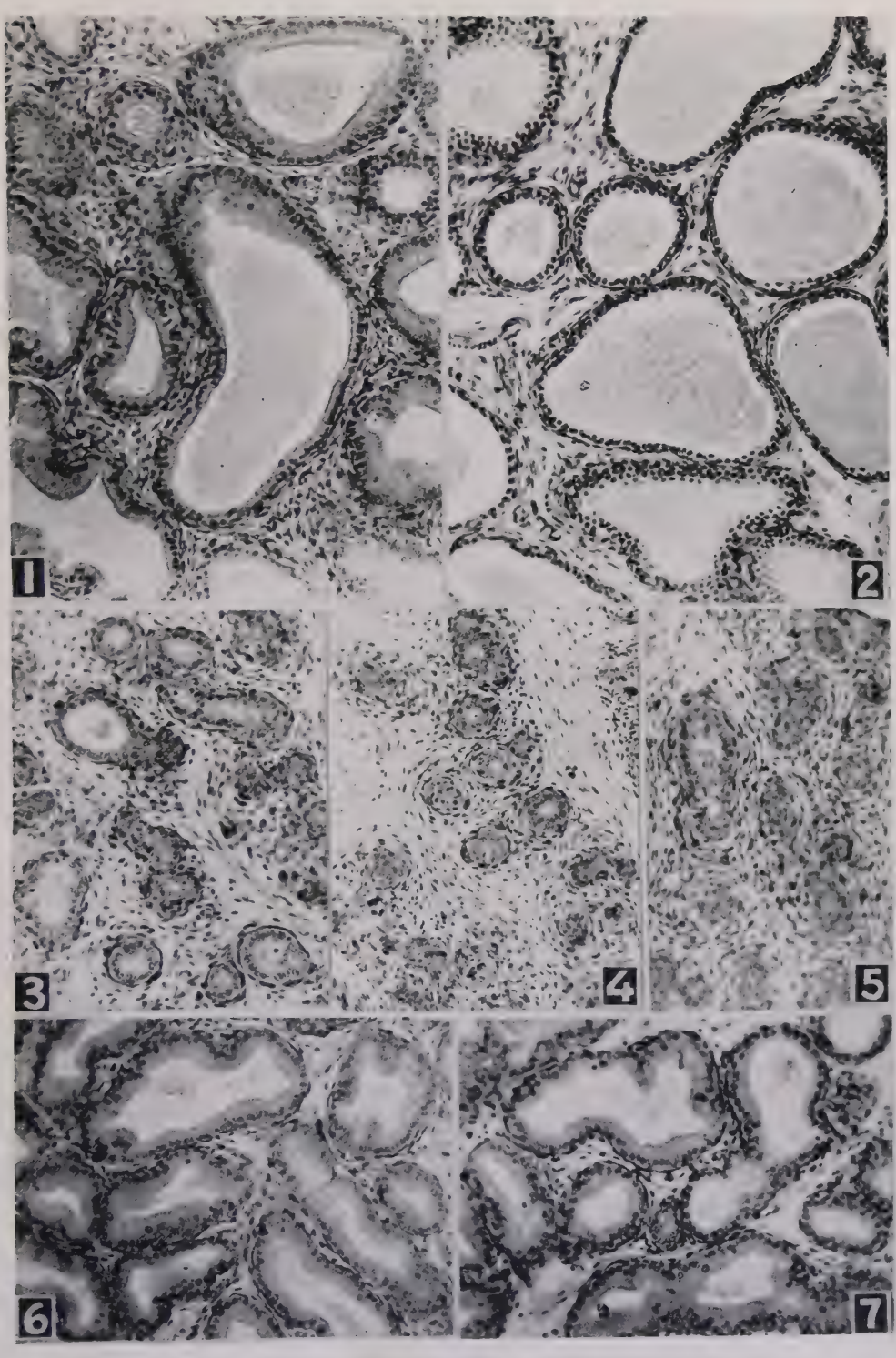
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PLATE 2

EXPLANATION OF FIGURES

8 Uterus of a 14-day-old normal female (mother spayed). The endometrium and myometrium are well developed and the glands penetrate to the base of the endometrium. Weight 15.4 mg.

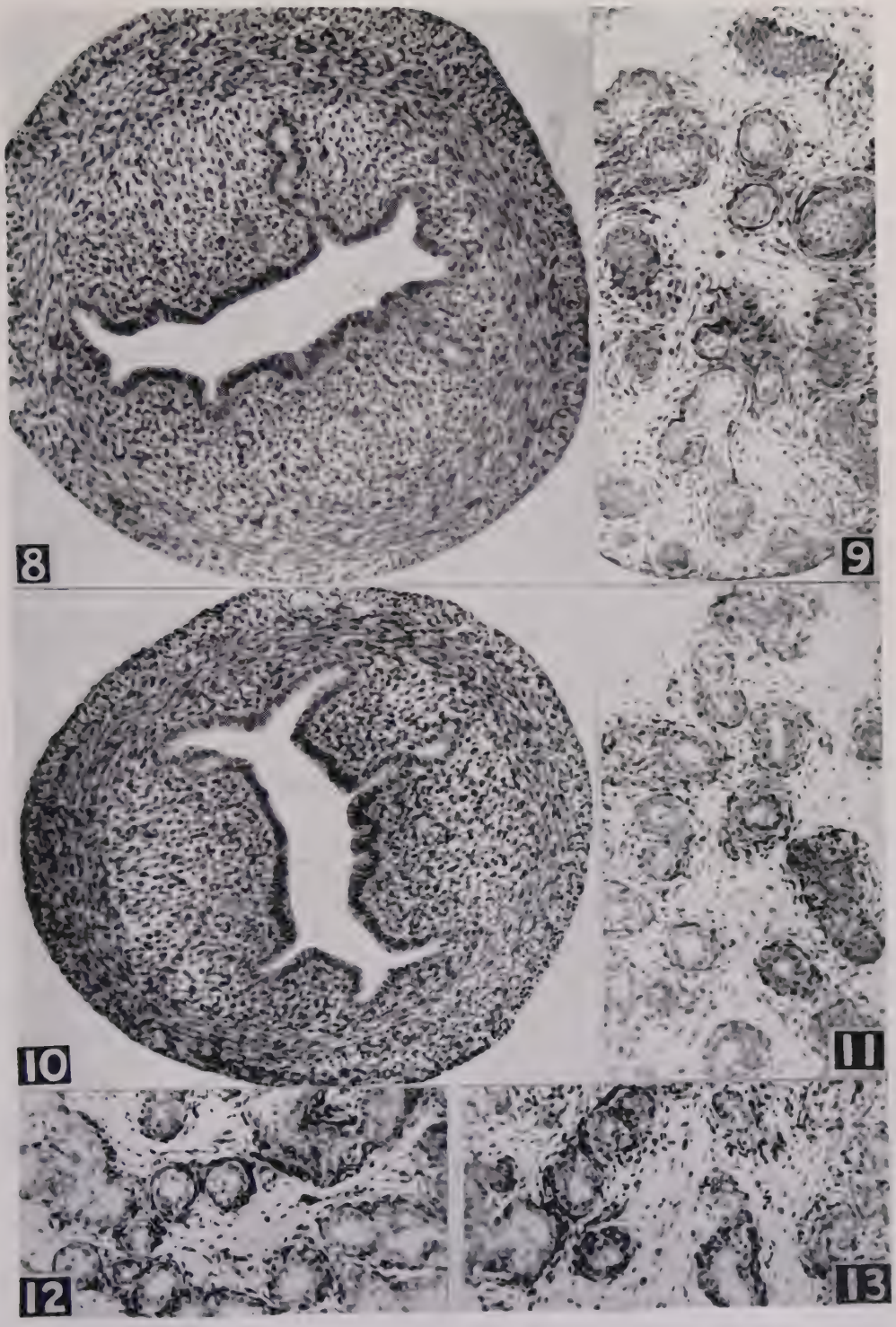
9 Prostate gland of a 14-day-old castrated male (mother normal). Lumina are beginning to form in the acini. Weight 1.8 mg.

10 Uterus of a 14-day-old spayed female (mother spayed). Littermate to the normal female in figure 8. The endometrium, myometrium and glands are as well developed as in figure 8. Weight 10.8 mg.

11 Prostate gland of a 14-day-old castrated male (mother spayed). Littermate to the normal male in figure 7. Histologically the gland resembles that of the castrated male (mother normal) in figure 9. Weight 1.5 mg.

12 Prostate gland of a 14-day-old normal female (mother normal). Lumina are beginning to form in the acini. Weight 0.6 mg.

13 Prostate gland of a 14-day-old spayed female (mother normal). Littermate to the female in figure 12. The gland appears essentially similar to the one in figure 12 but the lumina were not so well developed and there were more small acini. Weight 0.5 mg.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THE ACTION OF MALE AND FEMALE SEX HORMONES ON THE ADRENALS IN THE FOWL

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THREE FIGURES

THE PROBLEM

A great amount of work has been done on the influence of sex hormones on the adrenals of mammals but, unfortunately, their influence on the adrenals of birds has not received, as yet, the attention they deserve. However, Miller and Riddle ('39) have demonstrated that estrone stimulates the adrenals of the pigeon. Munro and Kosin ('40) observed that the adrenals of the female chicks were depressed due to treatment with estradiol or estradiol dipropionate. Bates et al. ('40) did not obtain any enlargement of the adrenals of cockerels after injection of estrin. Breneman ('40) on the other hand, reported hypertrophy of the adrenals of cockerels after administration of diethylstilbestrol and depression due to treatment with testosterone propionate. There is a great deal of discrepancy in the literature dealing with the action of sex hormones on the adrenals of mammals. According to one group of workers, the female sex hormone stimulates the adrenals. A second group, on the other hand, emphasizes its depressing effect. Most of the workers, however, are in agreement with regard to the depressing action of the male sex hormone on the adrenals. Since Ingle ('42) and Burrows ('45) have given extensive bibliographic reviews on the mammalian

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adrenals, citations to literature on that topic are held at a minimum in this report. However, in a review of the scant literature dealing with the action of sex hormones on the avian adrenals, the same diversity of results is evident. Further, all the workers in the avian field have used only immature animals in their experiments and as a consequence there is a great paucity of information regarding the action of sex hormones on the adrenals of adult animals. Moreover, none of them have described the histology of the adrenals after hormonal treatments. The present investigation was therefore attempted to fill these gaps. Special attention in this study has been paid to the histological changes elicited by the sex hormones on the adrenals.

EXPERIMENTAL PROCEDURE

All the birds used in this experiment came from the pure bred Brown Leghorn flock of the Institute. A total of 30 females, 22 months old, were involved in this study. Out of the total of 30 birds, 10 served as controls, 10 received diethylstilbestrol and the remaining 10 were injected with testosterone propionate. It has been assumed that the size of the adrenals is roughly proportional to the size of the body, and for this reason the birds were carefully selected for uniform body size (2270 gm) before the commencement of the experiment. The controls and the injected birds were kept under identical husbandry conditions throughout the duration of the experimental period.

Diethylstilbestrol was dissolved in 70% alcohol (4 mg to 0.2 cm^3 of alcohol). This alcoholic solution was diluted with 1.8 cm^3 of 0.9% physiological saline solution with the result that the final solution contained 2 mg of diethylstilbestrol per cm^3. This hormone-impregnated alcohol plus saline solution was then injected at the rate of 2.5 cm^3 daily into the pectoral muscle of the birds (table 1).

Testosterone propionate was dissolved in arachis oil (110 mg to 22 cm^3 of oil) by heating gently over a flame.

One cm^3 of this solution containing 5 mg of testosterone propionate was then injected daily into the pectoral muscle of the birds (table 1).

The birds were autopsied on the day following the last injections. The adrenals were dissected out, weighed to the nearest mg and finally fixed in 10% formol-saline solution for histological study. Paraffin sections 8 microns thick were prepared in the usual way and were stained with Harris' haematoxylin followed by alcoholic eosin.

The experimental part of this investigation was conducted at the Institute of Animal Genetics, Edinburgh, during the summer of 1946, and the histological part was carried out at the Department of Poultry Husbandry, Cornell University, Ithaca, New York.

RESULTS

Weight

The adrenal weight of birds treated with diethylstilbestrol was similar to that of the controls and the same was also true of the birds injected with testosterone propionate (table 1).

TABLE 1

The effect of hormonal treatments on the adrenal weight.

DAILY TREATMENT	NO. OF BIRDS	AGE OF THE BIRDS AT COMMENCEMENT OF TREATMENT	EXTENT OF INJECTION FERIOD	TOTAL QUANTITY OF HORMONE INJECTED	AVERAGE ADRENAL WEIGHT
mg		*months*	*days*	*mg*	*mg*
Uninjected	10	22	..	...	101.8 ± 0.9 [1]
Diethylstilbestrol (5)	10	22	22	110	101.7 ± 0.5
Testosterone propionate (5)	10	22	22	110	101.1 ± 0.6

[1] Standard error of the mean was used throughout.

Histological

Controls. The terms cortex and medulla which are used in the descriptions that follow refer only to the well known types of tissue, but not to position in the gland. In the fowl the

cortical tissue is distributed throughout the gland in the form of irregularly arranged columns frequently anastomosing with each other and interspersed with groups of medullary cells. These columns of cortical tissue have been referred to as the "Chief strands" because they appear to form the foundation for the other elements of the gland (Grollman, '36). The cortical masses are uniform throughout the gland and not larger in the periphery as in the case of the pigeon (Miller and Riddle, '42). Each column of cortical tissue consists of cells which differ in their form. Thus round, oval, ovoid, hexagonal or even octagonal cells are encountered in the cortex. Each cell has many rounded vacuoles of different sizes which form a very conspicuous feature of these cells (fig. 1). The vacuoles indicate the positions which in a living cell are occupied by lipoid granules which however, have been dissolved out during the process of dehydration and clearing. The average diameter of the largest vacuole is found to be 4.7 ± 0.3 microns. In mammals similar vacuolated cells are encountered in the fascicular zone of the adrenals and owing to their spongy appearance after the usual histological procedures they are often referred to as the "Spongiocytes" (Smith and Copenhaver, '44). The cytoplasm of the cortical cells is mostly aggregated round the nucleus and the periphery. The nuclei are rounded or oval in form and contain scattered chromatin granules. The location of the nucleus in a cell is central.

The medullary tissue has no regular arrangement. It occurs mostly in masses of 2 to 3 up to 30 to 40 cells. These masses have been referred to as the "Intermediate strands" (Grollman, '36). Between the masses of cortical and medullary tissue there is an intricate network of blood sinusoids. There is a tendency for the cortical strands to be composed of a double row of cells with their long axes in the transverse plane of the strand. One end of the cells composing such a strand faces on the blood sinusoids. It might be mentioned here that a similar condition of the cortical strands has been described in the pigeon (Miller and Riddle, '42).

Diethylstilbestrol treated. The gross histological features of the cortical strands and the arrangement of the component cells are similar to those of the controls. However, a slight increase in vascularity is observed throughout the gland. This is evident from the presence of small hemorrhagic areas which are not encountered in the controls. The "Ring effect" induced in the adrenal cortex of mice due to diethylstilbestrol treatment (McPhail and Read, '42) is not seen in the fowl. The cortical cells appear to be more vacuolated and the average diameter of the largest vacuole is found to be 5.5 ± 0.2 microns which is greater than that of the controls.

No changes could be detected in the medullary tissue.

Testosterone propionate treated. The cortical cells appear slightly taller and narrower than either those of the controls or the estrogen treated group. The cells exhibit extremely minute vacuoles, the largest one of which averages 0.9 ± 0.09 microns in diameter. Owing to the smallness of the vacuoles the cells have lost the characteristic spongy appearance (fig. 3). Unlike that of the diethylstilbestrol treated group, hemmorhagic areas are not encountered in the gland.

Histological features of the medullary tissue are found to be the same as in the controls.

DISCUSSION

With regard to the effect of diethylstilbestrol treatment on the adrenal weight, the results obtained here are somewhat at variance with those obtained with chicks by Breneman ('42) and Munro and Kosin ('40) and with pigeons by Miller and Riddle ('39). Freudenberger and Clausen ('37), Selye et al. ('35), Mellish et al. ('40) and Grollman ('36) did not find any effect of estrogen treatment on the adrenal weight of rats and in this respect the present results are not out of keeping with those obtained by these workers. Regarding the lack of action of testosterone propionate, the present studies appear to be in disagreement with those on chicks by Breneman ('42) and mice by Selye ('39). However, Peczenik ('44) has reported a

similar lack of effect of testosterone propionate on the adrenal weight of golden hamster.

The lack of effect of diethylstilbestrol on the adrenal weight can be explained by assuming that the female sex hormone does not influence the adult adrenal in the females. Similarly the inability of testosterone propionate to influence the adult adrenals in the female can be accounted for by the assumption that they are not influenced by the male sex hormone. Since male birds were not involved in this study the later statement must be regarded as tentative, subject to revision on the acquisition of further experimental data.

It is well known that poisons and other unfavorable influences bring about marked hypertrophy of the adrenals. The mechanism involved is that under such conditions unusual amounts of corticotropic hormone is liberated by the pituitary (vide Ingle, '42). It has been suggested that treatment with diethylstilbestrol is another such non-specific influence which eventuates in the enlargement of the adrenals (Smith, '42). Since diethylstilbestrol failed to stimulate the adrenals in the present studies it would appear that the effect was perhaps a specific hormonal one and consequently the adrenal weight was not influenced. However, this subject is still controversial as is apparent from the papers of Allen and Bern ('42) and Bates et al. ('42). Perhaps the acquisition of more experimental data will clarify the situation.

Diethylstilbestrol treatment resulted in an increase in size of the vacuoles in the cortical cells. Since a vacuole represents the site occupied by a lipoid granule in the unprocessed cortex which has been dissolved out during the technical procedure employed in this study, it can be assumed that the increase in size of the vacuoles represents an increase in the lipoid content of the cortex. Vicari ('43) has also shown that an increase in the cortical lipoids occurs with estrogens in rats. However, it has been satisfactorily established that the administration of exogenous estrogens causes a rise in the lipoid level of blood in the fowl (Lorenz et al., '38; Zondek and Marx, '39). In the light of this finding it can be assumed that

the administration of diethylstilbestrol to fowls in the present studies also resulted in an increase in the lipoid level of the blood. Perhaps this rise in the lipoid content of the blood was also responsible for a simultaneous rise in the lipoid level of different tissues and organs which probably accounts for the increase in the lipoid content of the cortex as suggested in this study.

Testosterone treatment caused a decrease in the size of the cortical vacuoles. Taking the size of the largest vacuole in a cortical cell as the criterion, it can be assumed that testosterone has ensued a decrease in the lipoid level of the cortex. Korenchevsky et al. ('41) reported that testosterone propionate treatment caused heavy deposition of fat in the abdominal region of rats. Perhaps it acted in a similar manner in the fowl and caused mobilization of excess fat in the abdominal region which probably caused a demand on different sources rich in lipoid matter. It is not unlikely that the adrenal cortex played a part in meeting this general demand and the resultant effect of this was a decrease in its lipoid level.

The increased vacuolization of the cortex after administration of exogenous estrogens has been reported by a number of workers in the mammalian field (vide Ingle, '42). The present studies show that this phenomenon occurs also in the case of the fowl. This increase or decrease in vacuolization of the cortical cells due to hormonal treatment and the probable changes in the lipoid content of the cortex as suggested in this paper raises the question of the relationship between the lipoid level of the cortex and its functional activity. Flexner and Grollman ('39) suggested that the lipoid content is low when the functional activity of the cortex is low and is increased when the secretory activity is supposed to be increased. Although the proof of this statement is lacking, it is suggestive, but until further experimentation is conducted it will remain unsubstantiated. However, it might pointed out here that an important factor of this aspect of adrenal physiology which so far has received only scant attention from the workers is the role played by the pituitary. There is definite evidence that

the adrenals are under the hormonal control of the pituitary. Thus the atrophy of the cortex rapidly follows hypophysectomy which can be restored by the adequate administration of the corticotrophic hormone of the anterior pituitary (Cameron, '45). It appears probable therefore that a detailed elucidation of the adreno-hypophyseal relationship with special reference to animals treated with exogenous sex hormones might throw a flood of light on the functional activity of the cortex to its lipoid level.

The present investigation is of significance in that here adult birds have been used while all the work in this field has been done with immature birds. It furthermore adds in some respects to the evidence that has accumulated in this field in which there has been much diversity of opinion.

SUMMARY

1. Injection of diethylstilbestrol into adult female fowls failed to produce any effect on the adrenal weight. This treatment caused an increase in size of the vacuoles of the cortex.

2. Testosterone propionate treatment was without any effect on the adrenal weight of adult female fowls. A decrease in size of the vacuoles of the cortex ensued as a result of this treatment.

3. The probable significance of the increase or decrease in size of the cortical vacuoles after hormonal treatments has been discussed.

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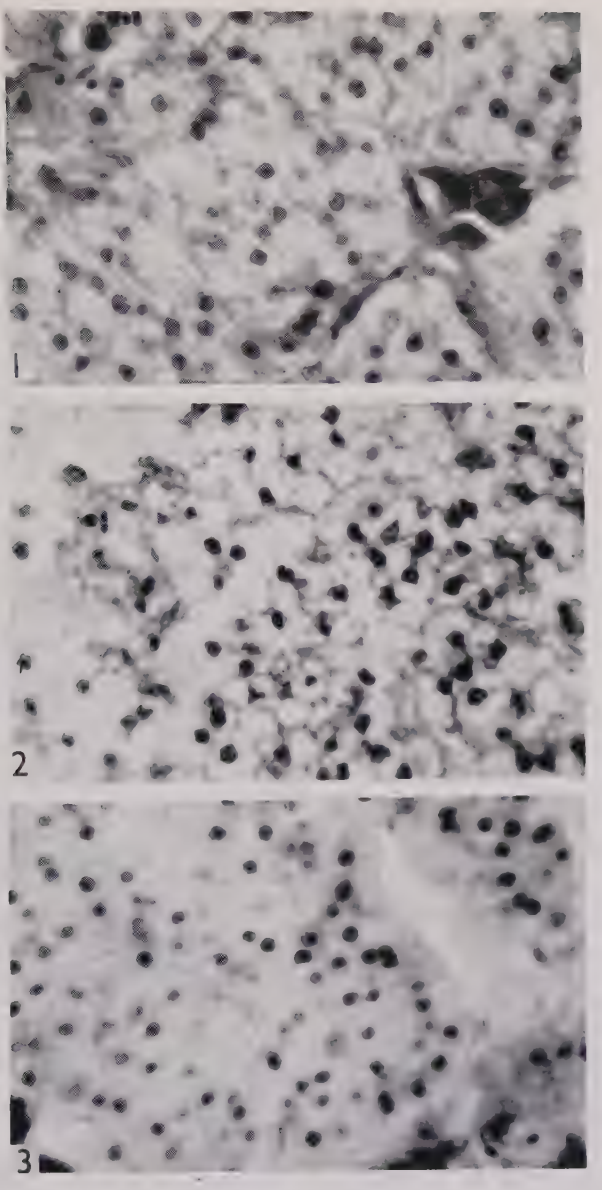

(All figures are magnified × 130)

1 Photomicrograph of the section through the adrenal of uninjected adult female fowl.

2 Photomicrograph of the section through the adrenal of adult female fowl injected with diethylstilbestrol. Note the large size of the cortical vacuoles.

3 Photomicrograph of the section through the adrenal of adult female fowl injected with testosterone propionate. Note the extreme small size of the cortical vacuoles.

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